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RELATIONSHIPS BETWEEN NUTRIENT COMPOSITION AND METABOLIC ENERGY DETERMINED WITH ENZYME AND GAS TECHNIQUE IN FEED SOURCES

Çerçi, İ. H., Tatli Seven, P.
Azman, M. A., Birben, N.

The University of Firat, Faculty of Veterinary Medicine
Department of Animal Nutrition and Nutrition Diseases, 23119, Elazig
Turkey

pintatli@hotmail.com

ABSTRACT

This study was conducted to find the effect of the nutrient which is related to the metabolizable energy of feed, including crude protein, crude fiber and nitrogen-free extract determined with enzyme and gas techniques. The investigation was carried out with 35 concentrated feed: oat (5), barley (5), wheat (5), maize (5) and soybean meal (5), cottonseed meal (5), sunflower meal (5), and 5 alfalfa hay and 5 grass hay. All the 35 samples were collected from different regions in Turkey. For determining the energy levels of feeds gas technique was used. Relationships between metabolic energy levels and components crude protein, crude fiber and nitrogen free extract of feeds were determined. Correlations between crude protein, crude fiber levels and metabolic energy in enzyme technique for forages, protein sources were found at $r = -0.99$ ($P < 0.01$), $r = 0.55$ ($P < 0.05$); $r = 0.99$ ($P < 0.01$), $r = -0.68$ ($P < 0.01$), respectively. Correlations between crude protein levels and metabolic energy in gas technique for forages, protein sources and grains and were found at $r = -0.73$ ($P < 0.05$); $r = 0.90$ ($P < 0.01$) and $r = 0.04$ ($P > 0.05$), respectively.

Key words: enzyme technique; gas technique; metabolic energy

INTRODUCTION

The prediction of the quality of feeds is important for the prediction of animal performance. *In vivo* measurements are expensive and there are difficulties associated with the procedures. For these reasons, differing techniques have been developed. The energy value of feeds can be determined by enzymatic methods, which do not require rumen fluid. The digestibility of feeds is generally related to just one chemical component or fraction of the feed. However, observed digestibility and metabolic energy values will be lower than the potential maximum because of the escape from the rumen of potentially digestible feed (11). The *in vitro* gas production technique has been improved as a predictive tool by which the kinetics of fermentation can be assessed (12). Relationships have been observed between a feed's gas production profiles and *in vivo* parameters, such as energy value and feed intake (11).

The digestibility and metabolizable energy value of feedstuffs are controlled by many chemical factors. This loss of potentially digestible material depends on animal species, physical form of the feed and the ability of the feed and animal supply the elements required by the rumen micro flora for maximum digestion. The loss of potentially digestible material is relatively small so it is unlikely to invalidate any conclusions regarding the effect of chemical composition on the digestibility and metabolizable energy value of feedstuffs (12). For many purposes quicker and cheaper laboratory methods of predicting digestibility are required. *In vitro* methods (16, 3) usually predict *in vivo* digestibility with a lower error than any chemical method.

The objective of the present work was to investigate the effect of some nutrients, which are related to the metabolisable energy of feed, including crude protein, crude fiber and nitrogen free extract with enzyme and gas techniques.

MATERIAL AND METHODS

Feed Samples and Chemical Analysis

The investigation was carried out with 35 concentrate feed: oat (5), barley (5), wheat (5), maize (5) and soybean meal (5), cottonseed meal (5), sunflower meal (5), 5 alfalfa hay and 5 grass hays. All the 35 samples were collected from different regions in Turkey.

Feed samples and *in situ* samples were analyzed for dry matter (DM), crude protein (CP), ether extracts (EE) and ash by the Weende methods as described by AOAC (1), and crude fiber (CF) as described by (4).

In vitro Enzymatic Technique

In this study the enzymes cellulose (*Trichoderma viride*, Sigma C-9422), hemicelluloses (*Aspergillus niger,* Sigma H-2125) amylase (extracted porcine pancreas, Sigma A-3176) and pepsin (Merck, 7190-2000 FIP-U/g) were used. All procedures were carried out according to D'orleans *et al.* (6), Aufrere (2).

In vitro Gas Production Technique

Rumen liquor was obtained from three ruminally fistulated sheep (two years old), maintained on a 900 gram good quality alfalfa hay and 300 gram concentrate diet according to their requirements. The rumen fluid preparation procedure and the composition of the buffer solution have been described by Menke and Stingass (10). All laboratory handling was carried out under continuous flushing with CO_2. All incubations were duplicated and completed in 100 ml calibrated glass syringes. The incubations were carried out with approximately 200 mg feed samples (DM) according to Menke and Stingass (10) and gas production was recorded at 24 hours.

Statistical Analysis

The computer programmer SPSS for Windows (Release 6.0, Spss inc. 1993) was used for regression procedures. The coefficients or correlation (r) of the estimate were calculated as they indicate the strength of the association and the accuracy of the equations respectively.

RESULTS

Correlations between crude protein, crude fiber levels and metabolic energy in enzyme technique for forages, protein sources were found at $r = -0.99$ ($P < 0.01$), $r = 0.55$ ($P < 0.05$), $r = 0.99$ ($P < 0.01$), $r = -0.68$ ($P < 0.01$), respectively.

Correlations between crude protein levels and metabolic energy in gas technique for forages, protein sources and grains and were found at $r = -0.73$ ($P < 0.05$), $r = 0.90$ ($P < 0.01$) and $r = 0.04$ ($P > 0.05$), respectively (Table 2). Correlations between crude fiber levels and metabolic energy in gas technique for forages, protein sources and grains and were found at $r = 0.72$ $P < 0.05$, $r = 0.94$ ($P < 0.01$), $r = 0.21$ ($P > 0.05$), respectively (Table 2). Correlations between nitrogen free extract levels and metabolic energy in gas technique for forages, protein sources and grains and were found at $r = 0.70$ ($P < 0.05$), $r = 0.75$ ($P < 0.01$), $r = 0.21$ ($P > 0.05$), respectively (Table 2). Correlations between enzyme technique and gas technique for forages, protein sources and grains and were found at $r = 0.76$ ($P < 0.05$), $r = 0.85$ ($P < 0.01$), $r = 0.16$ ($P > 0.05$) (Table 3).

DISCUSSION

In this study, a negative relationship was determined between protein levels and metabolic energy in forages using the enzyme technique (Table 2). This may be because crude protein is not an accurate predictor of available energy in forages because it constitutes a relatively small fraction (5 to 25) of the total forage, and the variability in the digestibility of the non-protein frac-

Table 1. The chemical composition of the feeds (DM %)

	n	Dry matter (DM)	Ash	Organic matter	Crude protein	Crude fiber	Ether extracts (EE)	Nitrogen free extract
Oat	5	91.33 ± 1.42	3.89 ± 0.74	96.11 ± 0.73	10.39 ± 3.64	11.52 ± 1.75	5.42 ± 1.41	68.78 ± 4.15
Barley	5	90.32 ± 0.68	2.57 ± 0.40	97.43 ± 0.40	11.22 ± 0.99	4.70 ± 1.53	2.88 ± 1.06	78.63 ± 1.33
Maize	5	89.71 ± 0.35	1.73 ± 0.36	98.27 ± 0.39	10.38 ± 2.97	5.67 ± 1.51	3.96 ± 1.02	78.26 ± 0.95
Wheat	5	90.03 ± 0.97	4.49 ± 0.96	95.50 ± 1.10	9.97 ± 1.51	3.20 ± 1.22	2.37 ± 0.94	79.96 ± 1.54
Alfalfa	5	92.71 ± 0.70	10.50 ± 1.61	89.50 ± 1.59	19.97 ± 1.18	23.13 ± 1.22	5.09 ± 1.20	41.31 ± 1.41
Grass Hay	5	92.27 ± 2.46	9.86 ± 1.15	90.14 ± 0.72	12.96 ± 1.99	30.64 ± 0.56	2.93 ± 1.30	43.60 ± 1.20
Soybean Meal	5	90.68 ± 1.17	6.28 ± 1.85	93.71 ± 1.18	52.75 ± 2.10	8.13 ± 1.11	4.43 ± 1.23	28.40 ± 2.40
Cottonseed Meal	5	94.36 ± 1.41	5.28 ± 1.52	94.72 ± 1.25	30.79 ± 1.32	22.02 ± 1.55	6.34 ± 1.41	35.65 ± 1.22
Sunflower Meal	5	93.31 ± 0.89	6.36 ± 0.96	93.64 ± 1.02	24.94 ± 1.20	23.60 ± 0.94	3.20 ± 0.85	41.90 ± 1.63

Table 2. Relationships between crude protein (CP) crude fiber (CF) nitrogen free extract (NFE) contents and metabolic energy (Y) of feedstuffs in enzyme and gas techniques (DM)

Feeds	*In vitro* enzyme technique Simple regression	Correlation	P	*In vitro* gas technique Simple regression	Correlation	P
Forage Sources	Y(ME) = 2377.426 – 38.23 CP	– 0.999	P < 0.01	Y(ME) = 2128 – 2.14 CP	– 0.728	P < 0,05
	Y(ME) = 789.534 + 35.65 CF	0.998	P < 0.01	Y(ME) = 1244 + 1.974 CF	0.719	P < 0,05
	Y(ME) = –3194.1 + 116.41 NFE	0.995	P < 0.01	Y(ME) = – 893 + 6.28 NFE	0.699	P < 0,05
Protein Sources (n = 15)						
	Y(ME) = 1732.27 + 2.59 CP	0.55	P < 0.05	Y(ME) = 1970.79 + 5.14 CP	0.903	P < 0,01
	Y(ME) = 1921.05 – 5.14 CF	– 0.68	P < 0.01	Y(ME) = 2313.82 – 8.51 CF	– 0.943	P < 0,01
	Y(ME) = 1932.56 – 3.01 NFE	– 0.29	P > 0.05	Y(ME) = 2483.2 – 9.24 NFE	– 0.749	P < 0,01
Grain Feeds (n = 20)						
	Y(ME) = 2014.23 – 17.89 CP	0.140	P > 0.05	Y(ME) = 2387.86 + 18.56 CP	0.045	P > 0,05
	Y(ME) = 1828.70 – 0.31 CF	0.015	P > 0.05	Y(ME) = 2458.59 + 14.60 CF	0.212	P > 0,05
	Y(ME) = 1854.84 – 0.39 NFE	0.03	P > 0.05	Y(ME) = 3328.02 – 10.04 NFE	0.212	P > 0,05

tion can be high (17). Fonnesbeck *et al.* (7) have reported that crude protein was not a good predictor of available energy in concentrates. Gas production was affected by differences in nutrient matter included in feedstuffs.

In this study, a negative correlation ($P < 0.05$) between metabolic energy and crude protein content for forage by gas technique was found. Iantcchheva *et al.* (9) have reported that in alfalfa hays, gas production is poorly correlated with *in vivo* or *in vitro* digestibility parameters. They explained that the reason for this is that the amount of gas produced is reduced by the formation of NH_4HCO_3 when NH_3 is liberated from protein degradation. Hence, the amount of NH_3 varies considerably in these samples (especially in alfalfa hays). Variability in chemical composition and available energy content usually is much greater for forages than concentrates (Weiss, 17).

In this study, a significant relationship was found ($P < 0.05$) between fiber, nitrogen free extract contents of feeds and metabolic energy levels, but there were no significant relationships ($P > 0.05$) in grains. This may be due to grains having similar nutrient constituents (17). Fiber is the most common variable used to predict the energy content of feeds (13). A negative relationship between fiber content and available energy occurs (17). This opinion agrees with our study (for only protein sources).

Lavrencic *et al.* (8) have confirmed that, with ryegrass and tall fescue hays incubated in the rumen, there is a close association between the chemical composition and the time of maximum degradation rate, suggesting that the structural arrangement and types of linkages within and between components regulate the fermentation process (14). In a study carried out by Tilley and Terry (16), enzyme and gas techniques were compared from the point of view cornstarch digestibility and a high correlation, ($r = 0.96$), was found between the techniques. But, Cone (5) has reported that a lower correlation ($r = 0.77$) has been found between the techniques when different enzymes were used in same study. In this study and our study it appears that changes in the relationship between techniques are connected with changes of feed source and enzyme used in the studies (Table 3).

Table 3. Relationships between enzyme and gas technique as metabolic energy (ME) determined for feedstuffs

Feeds	Techniques	Simple Regression	Correlation	P
Forages (n = 10)	Enzyme technique (ME)-Gas technique (ME)	Y(ME) = – 17.64 + 0,99 Gas ME	0.76	P < 0.05
Protein sources (n = 15)	Enzyme technique (ME)-Gas technique (ME)	Y (ME) = 292.294 + 0.71 Gas ME	0.853	P < 0.01
Grain Feeds (n = 20)	Enzyme technique (ME)-Gas technique (ME)	Y(ME) = 1951.10 – 4.84 Gas ME	0.16	P > 0.05

CONCLUSIONS

Generally significant relations between metabolic energy and feed components (crude protein, crude fiber, nitrogen free extract) with enzyme and gas technique were found. Grain feeds were not effective in the determining of the prediction of the quality of feeds. In this study, positive high relationships between enzyme and gas techniques in the forages and protein sources were often found.

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THE EFFECT OF CAFFEINE ON SELECTED METABOLIC PARAMETERS AND THE HEALTH OF CATTLE

Link, R., Kováč, G., Nagy, O.

II Clinical Department for Internal Diseases, University of Veterinary Medicine
Komenského 73, 041 81 Košice
The Slovak Republic

robolin65@hotmail.com

ABSTRACT

The aim of the trial was to determine haematological parameters, some enzymes and state of health after intravenous application of caffeine to six cows. Caffeine, at a dose 4.2 g, was administered to cows in *vena jugularis*. Their state of health was checked by clinical examination and blood was sampled before application and one, three, six and twenty-four hours after application. The temperature of the animals was within a normal physiological range during the whole period of the experiment. We observed a slight increase in temperature in the first hour. Heart rate decreased mildly over six hours, but twenty-four hours after administration it was same as at the beginning of the experiment. The intravenous application significantly influenced the haematological parameters in the first hour. The number of erythrocytes decreased from 6.5 $T.l^{-1}$ to 5.8 $T.l^{-1}$, which was statistically significant ($p < 0.05$), packed cell volume also decreased from 0.28 $l.l^{-1}$ to 0.23 $l.l^{-1}$ ($p < 0.01$). Three hours after application the number of erythrocytes increased to 6.11 $T.l^{-1}$ and the significant difference disappeared. The number of leukocytes and haemoglobin concentration was constant during the experiment. The activity of AST was not influenced by preparation. The activity of CPK was also in a normal physiological range, but at the 24th hour we found a significant decrease.

Key words: cows; caffeine; haematological profile; health state

INTRODUCTION

Caffeine, which belongs to the methylxanthines together with theobromine and theophylline, is 1, 3, 7-trimethylxanthine. Methylxanthines are partially demethyled and oxidised in the organism, 20 % of applied remedy is eliminated from organism in unchanged form. The methylxanthines are metabolised in the liver, it is important to be aware of eventual intoxication during liver disease. Intoxication with high doses of caffeine acts as excitation, convulses, restlessness. Caffeine is contraindicated for animals with diseases of the heart, where even a small dose can causes arrhythmia of the myocardium and excitations. On the other hand, caffeine is indicated for vasomotor collapse, in low doses for the better effect of analgesics and antipyretics (13). The toxic dose for a human being is approximately 10 grams of caffeine, it is estimated that average toxic dose for animals is 100 milligrams per kilogram of body weight (1).

The methylxanthines inhibit cyclic nucleotide phosphodiesterase and antagonise receptor-mediated actions of adenosine. These combined actions result in cerebral cortical stimulation and seizures, myocardial contraction, smooth muscle relaxation, and diuresis. Caffeine appears to stimulate the synthesis and release of catecholamines, especially norepinephrine. Caffeine stimulates the medullary, respiratory, vasomotor and vagal centers as well as the spinal cord at high doses. Skeletal muscles are stimulated more by caffeine than by other methylxanthines (7).

Caffeine acts as psychostimulans, in high doses increases reactivity, causes restlessness, and convulsions. However, the

effect is diminished by atropine and barbiturates, which decrease convulsions. In small doses caffeine enhances the attenuating reactions of brain that are utilised for a stronger effect of analgesics and antipyretics.

Vessels are dilated after the administration of caffeine, but also in this case the effect can be the opposite due to the stimulation of the vasomotor centre. Caffeine dilates coronary vessels and enhances the coronary blood stream. Muscular tissue is stimulated and its work is enhanced, even bronchi are dilated.

Two mechanisms increasing of urination are described:

a) Influence on renal circulation – as caffeine besides causing vasodilatation increases heart work. The blood stream in the kidneys is higher.

b) Re-absorption of natrium is slightly diminished and as a result osmotic concentration in papillae is also decreased.

The effect of caffeine on blood pressure is caused more by the effect on the vessel walls than direct effect on heart work. Caffeine is a non-selective antagonist of adenosine receptors A1 and A2. Adenosine receptor A1 inhibits the discharge of norepinephrine on the sympathic synapses of smooth muscles, and A2 receptors act as vasodilators. The important role of the aorta and big vessel walls is buffering changes in blood pressure in the veins and acts for a more continuous blood stream (11).

The contractions of the smooth muscles in vessel walls are initiated by calcium. This mineral element activates myosinkinase, which leads to muscle contraction. Consequently, calcium is the most important element in checking muscle contraction. Increased muscle contractibility after caffeine administration is caused by the increased entry of calcium and inhibition of sequestration by the sarcoplasmic reticulum (6).

R u e h l m a n n *et al.* (12) have reported on *vena cava* contractions after caffeine administration. He has found that caffeine increases calcium waves in the smooth muscle tissue of vessels. After caffeine administration the level of calcium increases and after 180 sit returns to the initial level. The same waves of calcium were observed also in cells, in spite of the constant presence of caffeine. As a result, contractions of tissue were in waves, the speed of contractions positively correlated with caffeine concentration.

F l y n n *et al.* (5) have described two types of calcium stocked vacuoles in the sarcoplasmic reticulum of smooth muscle cells. The first type contains only ryanodin receptor and therefore is sensitive to caffeine only in presence of ryanodin, and the second type contains also inozitol 1, 4, 5-trisphosphat receptor. The first type of stocked vacuole is filled with cytosolic calcium and the second one is filled with calcium from extracellular space. In the latter type, elimination of calcium from extracellular space emptied calcium stocks in vacuoles sensitive to inozitol.

The purpose of the work was to find the effect of caffeine on the general state of health and evaluate its influence on haematological profile and serum levels of AST and CPK.

MATERIAL AND METHODS

Six cows, lowland black spotted, at an age of four years, were included in the experiment. They were given 4.2 g of caffeine (10 % Coffeinum natrium, Biotika, Slovenská Ľupča, SR) intravenously as a sterile isotonic solution at the beginning of the trial. We evaluated its influence on general state of health – body temperature, breathing and heart rate, and also haematological profile and some parameters of enzymatic profile – activity of enzymes CPK, AST.

The state of health was checked by clinical examination and samples of blood for laboratory tests were collected before administration and then one, three, six and twenty-four hours after caffeine application. Haematological parameters were analysed by the automatic cell analyser SERONO 150 Plus (Switzerland), and the enzyme profile was analysed by the automatic analyser ALIZE (Lisabio, France), with the use of Bio Merieux kits (France). The results were analysed by Student's *t*-test.

RESULTS

Body temperature was in normal physiological range during the whole period of observation in all animals. In general, it was not influenced by caffeine, except the first hour after administration, when it slightly increased. Heart rate became slightly slower over six hours after application and it returned over twenty four hours to the initial. The number of breaths was in normal physiological range during the course of observation (Table 1).

Administration of caffeine had no side effects, and only in one case was mild excitation observed that lasted for three hours.

The activity of AST was not influenced by caffeine. It was stable in normal physiological range, which is from 1.3 to 2.2 μkat.l^{-1}, and only a slight decrease was found twenty-four hours after administration. Activity

Table 1. Body temperature, number of breaths and heart rate per minute in animals after caffeine administration

Parameter	0th hour	30th min	1st hour	3rd hour	6th hour	24th hour
Temperature (°C)	38.5 ± 0.2	38.6 ± 0.2	38.6 ± 0.1	38.6 ± 0.1	38.7 ± 0.1	38.6 ± 0.1
Breathing (min)	14.3 ± 2.9	16.5 ± 5.0	15.5 ± 3.3	15.7 ± 3.6	16.0 ± 3.5	16.5 ± 4.2
Heart rate (min)	50.3 ± 9.0	48.8 ± 8.6	50.3 ± 11.3	51.5 ± 11.0	50.5 ± 6.7	52.8 ± 5.8

Table 2. Activity of AST and CPK in blood of animals after caffeine administration

Enzyme	0th hour	1st hour	6th hour	24th hour
AST (μkat.l^{-1})	1.8 ± 0.70	1.7 ± 0.70	1.8 ± 0.60	1.5 ± 0.60
CPK (μkat.l^{-1})	5.7 ± 5.07[a]	5.5 ± 4.90	5.3 ± 3.50	3.4 ± 1.70[a]

AST — aspartate aminotransferase, CPK — creatine kinase, [a] — $p < 0.05$

of CPK was also in normal physiological range, which is up to 5.8 μkat.l^{-1}, but in this case we found a decrease within the 24th hour, which was significant compared with the 0th hour ($p < 0.05$). In general, CPK levels had a tendency to decrease during the period of the trial with the lowest activity twenty-four hours after application (Table 2).

Caffeine administration significantly decreased the number of erythrocytes (Er) ($p < 0.05$) and packed cell volume (PCV) ($p < 0.01$) within the first hour. These indices returned to the initial levels three hours after application and thereafter. Concentration of haemoglobin (Hb) slightly decreased but not significantly, the number of leukocytes (Le) was not influenced by caffeine (Table 3).

DISCUSSION

The increase in body temperature was mild, did not increase over the normal physiological range and was only temporary. This was expected because caffeine stimulates the circulatory, nervous and muscular system.

Methylxanthins inhibits the phosphodiesterase, that hydrolyses cyclic nucleotides. As a result, the concentration of intracellular cAMP increases. By this mechanism the stimulation of myocard and relaxation of smooth muscles can be explained. But it is not certain whether the concentration of caffeine *in vivo* is also so high as to be able to inhibit phosphodiesterase.

As a result of the inhibition of phosphodiesterase calcium is released. Consequently, in highly sensitive patients high doses of caffeine are able to cause heart arrhythmia, but in most cases it only enhances the strength and frequency of heart contractions. Methylxanthins are sometimes made use of for treatment of lung oedema, caused by heart weakness.

Caffeine causes stronger contractions of diaphragm and transversal-stripped muscles, which eliminate fatigue in patients with chronic obstruction of their lungs. This effect is probably the reason for improvement of the respiratory system in the course of hypoxia and dyspnoe, it can be observed also in cases of irreversible obstructions of the bronchi (11).

The activity of CPK and AST reflects internal processes in muscular tissues because CPK is important for the transport of ATP from mitochondria to sarcoplasmic reticulum. This transport of ATP helps the muscular tissues to work faster and more efficiently. It also helps to reabsorb the calcium discharged after triggering by caffeine, to the sarcoplasmic reticulum (3).

Mitochondrial isoenzyme CPK is bound to the outside of the internal mitochondrial membrane. It transphosphorylates ATP, which comes from mitochondria, to phosphocreatine. On the other hand, cytosolic isoenzym CPK, which is bound to myofibrils and sarcoplasmic reticulum uses phosphocreatine for re-phosphorylation ADP and this way supplies enough energy for muscle contraction or uptake of calcium to the sarcoplasmic reticulum (13).

K a a s i k *et al.* (10) have observed that in normal mice, ATP directly produced by mitochondria was not able to sustain calcium uptake and to relax rigor tension as efficiently as ATP produced by bound CPK.

After administration of caffeine *intra venam* to cows we observed a temporary decrease in the number of erythrocytes and consequently, a decrease in packed cell

Table 3. Haematological profile in animals after caffeine administration

Parameter	0th hour	1st hour	6th hour	24th hour
Er (T.l^{-1})	6.5 ± 1.10[a]	5.8 ± 1.36[a]	6.1 ± 1.25	6.3 ± 1.14
PCV (l.l^{-1})	0.28 ± 0.04[b]	0.23 ± 0.05[b]	0.25 ± 0.05	0.26 ± 0.04
MCV (f.l^{-1})	43.4 ± 3.20	41.0 ± 3.80	41.8 ± 4.20	42.6 ± 4.70
Le (G.l^{-1})	11.5 ± 5.00	9.53 ± 3.00	11.9 ± 5.98	11.1 ± 5.10
Hb (g.dl^{-1})	9.1 ± 1.00	8.9 ± 1.60	8.8 ± 1.30	8.3 ± 0.96

Er — erythrocytes, PCV — packed cell volume, MCV — mean corpuscular volume
Le — leukocytes, Hb — haemoglobin [a] — $p < 0.05$, [b] — $p < 0.01$

volume. But it improved over the course of three hours. Caffeine can influence vessels differently, according to individual reaction it is able to cause vasoconstriction or vasodilatation. If vasodilatation predominates, after the relaxing of the vasomotor nerves, packed cell volume decreases because part of the extracellular liquid comes to the blood stream. After this vasoconstriction occurs, blood pressure increases and the number of red blood cells improves spontaneously.

The effect of caffeine on the macroorganism is often connected with other matters such as starvation or water deprivation. They influence the length and strength of caffeine effect.

J a n u s *et al.* (9) have described that starvation was associated with an increase in the mean residence time of caffeine in each subject. The increase was statistically significant ($p < 0.01$). After starvation the total plasma clearance of caffeine decreased (about 20 per cent). The decrease was statistically significant ($p < 0.05$). Similarly, water deprivation was associated with a significant increase in the mean residence time of caffeine in each subject. The total plasma clearance of caffeine decreased statistically significantly ($p < 0.01$).

Clinical observation shows that men and old women had a higher incidence of diseases of the coronary vessels than young women. These observations suggest that estrogens can prevent vessels from vasoconstriction. C r e w s and K h a l i l (2) tested if the relaxation of vessels, which is caused by hormones, is a result of the inhibition of calcium mobilisation in smooth muscular tissues. They found estrogens significantly inhibit the contractions of coronary vessels, which was a result of calcium intake. The most important role in this inhibition was played by 17 β-estradiol, which prevents calcium entering the cells from extracellular space.

Caffeine concentrations in blood were closely positively related to triglycerides in caffeine-drug users (male: $p < 0.001$; $p = 0.018$). No associations have been found between caffeine concentrations and total cholesterol and LDL-C levels in any groups of D u *et al.* study (4).

The metabolism of caffeine is sexually different in ruminants at different ages. Caffeine, five mg per kg of body weight, was given intravenously as a sterile isotonic solution to Holstein cattle from age one month to age eighteen months. The experimental period was characterised by a steady decrease (statistically significant) in caffeine mean residence time (MRT). These values did not differ significantly between males and females under eight months of age. In eight-, twelve- and eigheen-month-old animals, the caffeine MRT in the females was significantly shorter than in the males. The total plasma clearance of caffeine increased significantly between one and eighteen months. No significant differences were observed between total plasma clearance of caffeine in males and females under eight months of age. In eight-, twelve- and eigheen-month-old animals, the clearence of caffeine was significantly higher in females than in males. In conclusion, a sex-linked difference in pharmacokinetics of caffeine was reported in cattle over eight months of age (8).

Our achieved results indicate that cows tolerate caffeine well after *intra venam* application. We observed only a slight excitation in one case, which disappeared in a short time, the circulatory and nervous system being stimulated to an appropriate degree.

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THE EFFECTS OF ORAL ADMINISTRATION OF IRON METHIONATE TO PREGNANT SOWS AND THEIR LITTERS

Petrichev, M. H., Bambova, M.*

Faculty of Veterinary Medicine, University of Forestry, Sofia
***Regional Veterinary Medicine Office, Blagoevgrad**
Bulgaria

metodipetrichev@yahoo.com

ABSTRACT

The aim of the study was to evaluate the effect of amino-acid chelated iron, administrated p. o. 20 days before farrowing, on the haematological indexes in sows and their litters. Twelve sows (cross bred Camborough) at one and the same time of gestation were used in the experiment. Twenty-one days before farrowing sows were randomly allotted among three groups and treated according to following design: the I group (n = 4) received twenty days experimental iron methionate (Fe = 14 %) with food in doses of 500 ppm, the II group (n = 4) were treated twenty days with the same product at a dose of 1000 ppm and the third group was a control. The erythrocyte count (Er), haemoglobin (Hb) concentration (on 0 and 10-th day), haematocrit (Ht) and leukocyte (Leu) count were measured. Sterile colostrum from each sow was taken on the day of farrowing for evaluation of the iron content. Blood samples from each piglet in the litters were taken 6 ± 1 hours after birth from the *sinus ophthalmicus*. In the same period the individual body weight of each piglet, Hb and Ht values, Er count and Leu count was evaluated. Samples from the liver and spleen (from one-two piglets from litters) were taken to estimate iron content. The results showed that treating the pregnant sows (twenty days before farrowing) with iron methionate benefits body weight and the number of newborn piglets. They have bigger iron deposit in their livers and spleens. The content of iron in the colostrum in treated sows is increased, too.

Key words: anaemia; iron methionate; pig

INTRODUCTION

The chelates, which is iron methionate, are inner-complex compositions, composed from a central atom (metal ion with $^{+}2$ valence or bigger valence), connected with two or more amino acid molecules with coordination bonds and formed heterocyclic rings.

A wide range of investigations has shown that using metal chelates like food additive provides better absorption of microelements, than using an inorganic form. This helps the increase of an animal's productivity and decreases fodder consumption for a unit of productivity. In the scientific literature positive results have been shown for trade chelate products "Super Fe-Max" (Borregaard Industries Ltd, Sarpsborg, Norway), "Bio-Plex" (Alltech, USA), "Availa-Fe" (Zinpro Co., USA). Darneley (6) and Parisini (15) have determined that inserting a different quantity iron in amino-acid chelate form in food, increases the number of newborn pigs (with 11/2 pig) from one sow, their repetition decreases fodder consumption necessary for 1 kilogram of weight gain.

The aim of this study is evaluation of antianemic effect in per oral treatment of pregnant sows and their litters with amino-acid chelated iron through last 20 days of pregnancy. In this period the foetus doubled its weight and the needs of iron are increased.

MATERIALS AND METHODS

Twelve sows, cross bred Camborough from a pig farm in Blagoevgrad (Bulgaria) were used in the experiment. Twenty-one days before farrowing they were divided into three groups. The pigs from the first group (n = 4) were treated until parturition with newsynthesized iron methionate (Fe – 14 %) added in their fodder at a concentration of 500 ppm. The pigs from the second group (n = 4) received iron methionate added to their fodder at a concentration of 1000 ppm for the same period. The third group was the control (without iron addition). Blood samples were collected from the *sinus ophthalmicus* before the experiment started –21 days before parturition (day 0), on the tenth day and just before farrowing. The Hb values and Er count were measured and Ht values and Leu count were measured only on the day before farrowing.

Sterile colostrums were taken from each sow for evaluation of the iron content in the milk on the day of farrowing. Blood samples from every piglet in the litters were taken 6 ± 1 hour after birth from the *sinus ophthalmicus*. The individual body weight of each piglet, Hb and Ht values, Er count and Leu count were evaluated. The haematological indexes were measured with an automatic haematological counter BC – 3000 plus Mindray. After collecting blood samples one to two piglets from litter were slaughtered after euthanasia and samples from the liver and spleen were taken for evaluation of the iron content with flame atomic-absorption spectrometry (Karl Zeiss Jena atomic absorption spectrophotometer-AAS 30).

The management of sows breeding and litters breeding was on a concrete floor with individual feeding and water. The sows were fed with a fodder mix containing a grain component barley – 43.50 %, sunflower cake – 30 %, bran –25 %, chalk – 1.20 %, and salt – 0.30 %. The piglets begin to fodder after the tenth day with a fodder mix, containing grain component maize – 40 % and wheat – 10 %; the protein was 20 % and energy – 13 MJ.

For the statistical analysis of the results a one-way analysis of variance (ANOVA) was performed.

The care and use of experimental animals observed the Law for protection and a humane attitude to laboratory animals (Instruction № 25/10. 06. 2003), Bulgaria.

RESULTS AND DISCUSSION

The results from the haematological investigations of experimental and control sows are shown on Table 1. It shows that there were no significant differences among haematological indexes in experimental sows (group 1 and 2) during the whole experimental period. But control sows indicated significant reduction in Hb and Er values on the day of farrowing. Many authors consider that sows with Hb value under 100 $g.l^{-1}$ are anaemic (10, 11, 13). With regard to this position in our experiment two control and three treated with iron methionate sows were anaemic on the day of farrowing. G u i s e and P e n n y (12) have proposed that a hormonal induced suppressive effect on the bone marrow can lead to normocytosis and hypochromic anaemia at the end of pregnancy. The mechanism of anaemia is not well known but there is an assumption that increased plasmatic levels of estrogens at the end of term are partly at the base of this process. Myelotoxicity of estrogens is firmly confirmed from several authors (4, 16). In sows an increase of free plasmatic estrogen activity in late pregnancy has been confirmed by D r a n e and S a b a (7) and A s h *et al.* (2).

The sows from the first group, treated with 500 ppm iron methionate delivered 42 live-born piglets where only two piglets were below one kilogram in weight i.e. with hypothrophic syndrome. The sows from the second group, received 1000 ppm iron methionate in the fodder, delivered 41 live-born piglets, where four of them were below one kilogram in weight. The sows from control group (untreated) delivered 38 live-born piglets, where five of them were below one kilogram in weight.

Table 1. Data on haematological indexes in sows

Group	Number	20 days before farrowing		10 days before farrowing		In the day of farrowing			
		Hb $g.l^{-1}$	Er $T.l^{-1}$	Hb $g.l^{-1}$	Er $T.l^{-1}$	Hb $g.l^{-1}$	Er $T.l^{-1}$	Htc %	Leu $g.l^{-1}$
I group. Treated with p.o. 500 ppm	4	105.63	4.31	109.25	3.13*	104.25	4.44	29.25	16.77
II group Treated with p.o.1000 ppm	4	103.30	4.11	106.53	3.14	103.00	4.46	30.75	14.73
III group Control	4	115.38	4.88	105.43	4.04	99.50	3.90	28.25	13.70

* — $P < 0.05$ Statistical significance according to controls

In Table 2 the results from haematological measures and body weights of pigs born from control and experimental sows are shown. As can be seen there is not a significant difference in haematological indexes in piglets born from sows of experimental and control groups. The body weight of piglets born from sows treated with iron methionate was influenced positively: in the second group after farrowing the newborns had an average body weight of 1.49 ± 0.09 kg. This data correlates with the results of Angelov (1).

Table 2. Data on haematological indexes and body weight in newborn pigs

Group	Number	Hb ($g.l^{-1}$)	Ht (%)	Er ($T.l^{-1}$)	Leu ($g.l^{-1}$)	BW (g)
I group	42	91.26*	24.69***	3.37	14.43	1420
II group	41	94.42	28.63	4.32*	11.83***	1490
III group	38	92.11	30.11	4.76	16.09	1410

* — P < 0.05; ** — P < 0.01; *** — P < 0.001
Statistical significance according to controls

Ashmead (3) has conducted similar experiment and he considers that the enhanced body weight and the higher per cent of survival in piglets, born from treated sows is because of amino-acid chelated iron supplementation.

The effect from iron methionate supplementation in sows' fodder on the iron content in liver and spleen of their litters (on the day of farrowing) is shown on Fig. 1. The iron amount in liver in piglets of Group I was 158.5 ± 41.47 ppm (p > 0.05); in control piglets – 136.2 ± 64.04 ppm, while in experimental Group II the values were significantly higher – 277.5 ± 81.82 ppm (p < 0.01) i.e. with 119.0 ppm higher than values from the first group and with 141.3 ppm higher than these of the control group. The differences are statistically significant (p < 0.01). The same tendency is observed in the iron content of the liver and spleen in the three groups of pigs (Fig. 1).

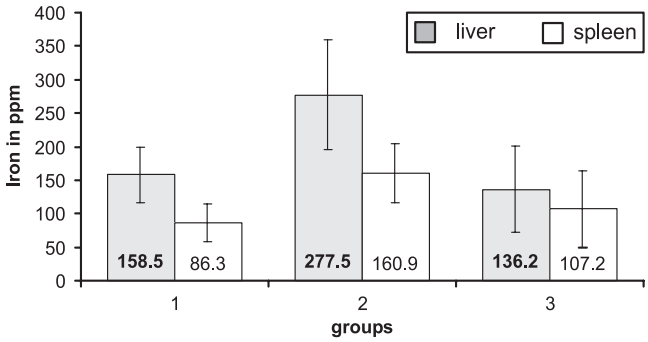

Fig. 1. Iron content (ppm) in liver and spleen

In Fig. 2 the results from iron content in sows' colostrums, treated with different doses iron methionate twenty days before farrowing (500 ppm and 1000 ppm iron) and for control sows (untreated) are shown. The highest iron level was in sows that had received iron methionate at a dose of 1000 ppm in the last three weeks of pregnancy (2.90 ± 0.89 ppm; p > 0.05). Sows treated with 500 ppm iron had iron content in their colostrums of 2.89 ± 1.17 ppm, while the control group had lower values (2.47 ± 0.69 ppm), but in the three groups there is no statistical significant difference (p > 0.05) – Fig. 2.

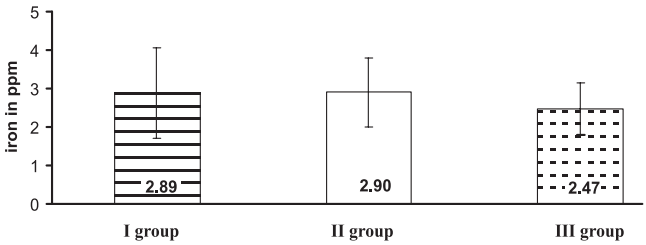

Fig. 2. Iron content (ppm) in colostrums

CONCLUSIONS

1. Inclusion of iron methionate in pregnant sows' ration twenty days before farrowing at doses of 500 ppm and 1000 ppm, leads to a positive influence on newborn piglets' weight and enhanced the number of live-born piglets.
2. In the body of newborn pigs more iron is accumulated in the liver of piglets born from treated sows – 277.5 *versus* 136.2 ppm in controls.
3. The iron in the colostrums of treated sows increases – 2.90 ppm *versus* 2.47 ppm in the control sows.

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GLUCOSE CONCENTRATION IN THE SERUM OF ARABIAN AND ANGLOARABIAN HORSES

Różański, P., Nowakowicz-Dębek, B., Saba, L.
Ondrašovič, M.*, Vargová, M.*

University of Agriculture in Lublin, Akademicka 13, 20-950 Lublin
Poland
***University of Veterinary Medicine, Komenského 73, 041 81 Košice**
The Slovak Republic

d.p.roza@wp.pl

ABSTRACT

Investigations were carried out at the Janów Podlaski Stud, Poland, from March 2001 to June 2002 focusing on serum glucose concentration in 88 Arabian and Angloarabian horses aged 14 ± 3 months. All the animals were kept under the same conditions and the necessary prophylactic measures were applied. Blood for examination was taken from the jugular vein prior to morning feeding. The study showed a difference between the breeds investigated, variations according to season and a slightly higher level of glucose concentration in the mares of both breeds.

Key words: Angloarabian; Arabian; horse; serum glucose

INTRODUCTION

For many years studies conducted at horse breeding and scientific institutions have focused on strengthening some desirable traits and passing them onto consecutive generations. Yet there is a vital problem concerning the recognition of the physiological differentiation of values of each individual parameter which is considered standard in the course of diagnostic procedures, therapeutic management and health status monitoring. This differentiation can constitute a common feature linking individual groups of horses, e.g. those of different breed, sex and age. The differences seem to be important enough to affect the interpretation of results of biochemical examinations and prophylactic-medicinal procedures significantly.

The objective of the present study was to determine the differences in serum glucose levels in Arabian and Angloarabian horses.

MATERIALS AND METHODS

The study was carried out at the Janów Podlaski Stud in the period from 2001 to 2002. It was organized as a series of six investigation, each lasting three months, starting mid-March 2001 (Ist sampling) and ending mid-June 2002 (VIth sampling). The animals investigated were Arabian and Angloarabian horses selected at random. At the time of the commencement of the examination the animals were 14 ± 3 months old. The investigated group consisted of 88 animals, 24 Arabian stallions and 33 Arabian mares and 13 Angloarabian stallions and 18 mares. Throughout the experimental period all the animals were under animal-breeder and veterinary care. The treatment and prophylactic measures were applied to all animals and were taken in due time. The maintenance conditions in the studs and the length of time spent in the run were the same. A group feeding system was used with feed produced on the farm. With regard to the quality and quantity of rations, the mineral composition of feed complied with the commonly accepted standards for horse feeding (6), as stated elsewhere (8).

All the additives in the form of mineral-vitamin preparations and carrot were available to all the horses in the same quantity and for the same time. Blood samples were collected from the jugular vein before morning feeding.

Table 1. Mean glucose concentration (for all samples and both sexes) (mmol.dm^{-3}) in blood serum of investigated horse breeds

	Parameter 00	Breed X0
n	129	127
$\bar{X}$	4.32^{a}	4.59^{b}
SD	0.071	0.085

00 — Arabian horses,
XO — Angloarabian horses,
n — group size, $\bar{X}$ — mean, SD — standard deviation
Mean values marked with different letters differed significantly:
a — P < 0.001; b — P < 0.05; c — P < 0.01

Table 2. Mean glucose concentration (for all the samples) (mmol.dm^{-3}) in blood serum depending on sex of the horses studied

Sex	Parameter	Glucose
♀	n	128
	$\bar{X}$	4.71^{a}
	SD	0.085
♂	n	128
	$\bar{X}$	4.19^{b}
	SD	0.071

n — group size, $\bar{X}$ — mean, SD — standard deviation
Mean values marked with different letters differed significantly:
a — P < 0.001; b — P < 0.05; c — P < 0.01

Table 3. Glucose levels (mmol.dm^{-3}) in blood serum in each investigation series

Parameter	Investigation series I	II	III	IV	V	VI
n	40	43	44	43	42	44
$\bar{X}$	3.91^{a}	4.71^{c}	4.69^{c}	4.82^{c}	4.54$^{b, c}$	4.04$^{a, b}$
SD	0.012	0.013	0.012	0.012	0.012	0.017

n — group size, $\bar{X}$ — mean, SD — standard deviation
Mean values marked with different letters differed significantly: $^{a, c}$ — P < 0.001; $^{b, g}$ — P < 0.05; $^{d, e, f}$ — P < 0.01

Table 4. Mean serum glucose levels in Arabian and Angloarabian horses (sum of mean values for both sexes) at each sampling

Breed	Parameter	Investigation series I	II	III	IV	V	VI
OO	n	19	21	23	23	22	21
	$\bar{X}$	3.63^{a}	5.26^{c}	4.76^{b}	4.78$^{b, c}$	4.34$^{a, b}$	3.34^{a}
	SD	0.017	0.019	0.016	0.016	0.017	0.017
XO	n	21	22	21	20	20	23
	$\bar{X}$	4.2$^{A, B}$	4.17$^{A, B}$	4.81^{B}	4.87$^{B, C}$	4.75$^{B, C}$	4.74$^{B, C}$
	SD	0.017	0.018	0.019	0.017	0.017	0.017

n — group size, $\bar{X}$ — mean, SD — standard deviation
Mean values (within breeds) marked with different letters differ significantly:
$^{a, b, e, f, g}$ — P < 0.001; $^{d, A, B}$ — P < 0.05; $^{c, D, E, F, G, H}$ — P < 0.01

Blood glucose levels were determined spectro-photometrically at 500 nm (Hg 546 nm) using CORMAY GLUCOSE – 500 LTS diagnostic kit complying with the manufacturer's recommendations.

The results obtained were analyzed statistically using two-factor variance analysis with repeated measurements, three-factor non-orthogonal variance analysis and multiple confidence intervals (*t*-Tukey's test). The computations were made using the following software: Statgraphic V. 5.0 and Statistica V.6.0.

RESULTS AND DISCUSSION

All the processes and reactions occurring in an organism represent a complex of changes termed the energy metabolism. Glucose is the principal energy source for every organism and its concentration in an animal body is affected by many factors and their mutual interactions. One important period of life when some significant deviations from the accepted standards are recorded are the first days in animal's life. According to R o s e *et al.* (7) it is necessary to consider this when

Table 5. Mean serum glucose levels(mmol.dm^{-3}) in horse breeds in relation to breed and sex

Sex	Parameter	Breed 00	Breed X0
♂	n	61	67
	$\bar{x}$	4.11[a]	4.28[a]
	SD	0.010	0.009
♀	n	68	60
	$\bar{x}$	4.53[a]	4.9[a]
	SD	0.009	0.014

n — group size, $\bar{x}$ — mean, SD — standard deviation
Mean values (within breeds) marked with different letters differ statistically: [a] — $P < 0.001$; [b] — $P < 0.05$; [c] — $P < 0.01$

interpreting the results of any studies. During this period glucose concentration in lactating mare undergoes changes that reflect turbulent hormonal processes and the secretion of milk. Analogical changes were also observed in blood serum where the glucose level was slightly lower but more stable (5).

Haematological parameters change over the first days of life and are related to very intensive adaptation processes in newborns. At this time, the organism of the young requires huge quantities of energy, the principal source of which is glucose. According to R o s e *et al.* (7), although glucose concentrations in foals vary considerably, they are maintained at higher levels compared to adult horses.

When analyzing serum glucose concentrations, it should be mentioned that Y a s h i k i *et al.* (12) have observed considerable diurnal variations, yet the results remained within the standard range. However, variations observed by these authors could hardly be regarded as standard because F l i s i ń s k a - B o j a n o w s k a *et al.* (1) failed to record them in their study conducted on foals and adult horses. The latter authors have described variations of serum glucose in foals only in the first period of their life and changes in their lactating mothers. K r u m r y c h and W i ś n i e w s k i (4) have shown the effect of sex on serum glucose concentration and indicated that higher levels of this parameter were detected in stallions as opposed to mares. Moreover, in Polish Primitive stallions some differences in glucose concentration were related to seasons (3).

Concentration of serum glucose changes over intensive training periods in association with physical effort. According to S z a r s k a (10) the blood glucose level in horses depends on many factors. Here we should mention the elevated concentration of cortisol and catecholamines induced by physical effort, which are responsible for the increase in glucose levels. Such assumption has not been confirmed by K ę d z i e r s k i and P o d o l a k (2) who investigated this serum parameter in Arabian horses outside the training period and observed that its concentration exceeded the standards recommended by S z a r s k a (10) and other authors (2). The levels given by K ę d z i e r s k i and P o d o l a k (2) were close to those mentioned by S z a r s k a in the horses at training (10).

The glucose levels presented in this study were within the limits defined as generally recognized reference values and confirmed by authors of other studies (10). The differences in glucose level found in the experimental animals were related to the breed as a higher concentration of this sugar was detected in Angloarabian horses (Tab. 1). When dealing with sex, higher values of glucose were characteristic of mare serum (Tab. 2). With regard to both considerations the difference was statistically significant.

The next factor examined in the present study was the influence of season (Tab. 3). Variations in serum glucose concentrations throughout the experiment fail to suggest an explicit effect of season on glucose levels in the investigated horses (Tabs. 3 and 4). However, the recorded fluctuations of glucose indicate that the stability of glucose concentration is evidently higher in the half-breed Angloarabian horses (Tabs. 3 and 4).

The stability observed, or its lack, seem to be independent on the diet. This statement is confirmed by the study conducted by S t e i n b r e c h e r (9) who did not find any correlation between the supplied feed and serum glucose level. K r u m r y c h *et al.* (3) did record seasonal variations in Polish Primitive horse and noted statistically significant differences. According to their results males dominated in this respect. Although considerably higher glucose levels were found in mares of the breeds investigated in our study, the differences were insignificant (Tab. 5).

Levels of individual minerals and biochemical constituents in blood serum may indicate some nutritional deficiency. M u r p h y (5) has stated that serum glucose concentrations are closely related to the diet and influenced by its composition.

All these opinions, concerning the relationship between feed composition and its influence on the concentration of serum components have one feature in common. It is a lack of agreement with respect to the characteristics of the examined groups of horses that may account for such a great diversity in results.

We should emphasize the fact that in spite of the relationships presented, the glucose level reflects an intensification of sugar metabolism as well as concentration of proteins and immunoglobulins and therefore it also indicates the intensity of the immune processes. Determination of glucose concentration as an energy source in animal bodies may help, in addition to other parameters, to assess the health status of new born animals even of adult animals in making a proper diagnosis (11). Yet, full application of this knowledge will only be possible when the study of relations between the breed, season, age and the level of glucose will provide additional detailed information.

CONCLUSIONS

The results obtained allow us to draw the following conclusions:

— observations showed differences in serum glucose concentration between the horse breeds studied,

— higher glucose levels were found in mares (mean of all samplings, and the examined individual samplings),

— variations in concentrations between individual sampling-seasons were observed with respect to mean values for all horses and within the breeds (disregarding the sex),

— with both breeds investigated blood glucose concentration was slightly higher in mares.

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LAMINOPLASTY A NOVEL SURGICAL TECHNIQUE FOR INTRASPINAL PROCEDURES IN DOGS

Šulla, I.[1], Vanický, I.[2], Danko, J.[1], Kafka, J.[3]

[1]University of Veterinary Medicine, Komenského 73, 041 81 Košice
[2]Neurobiological Institute of the Slovak Academy of Sciences, Šoltésovej 4, 040 01 Košice
[3]Department of Neurosurgery, University Hospital, Tr. SNP 1, 040 01 Košice
The Slovak Republic

sulla@uvm.sk

ABSTRACT

Authors report on a positive experience with an osteoplastic surgical approach to the spine, spinal cord and *cauda equina* in dogs. This surgical technique, originally elaborated by neurosurgeons with the aim of preventing the possible development of kyphotic deformities of the body axis in children and adolecents due to extensive laminectomies, was primarily tested on anatomical specimens of canine vertebrae, subsequently used during experimental operations on a group of nine adult mongrel dogs with very promising results. Short-term as well as long-term (maximum survival time of two animals was three months) observations showed, that laminoplasty is a suitable alternative method to a classical laminectomy for spinal operations in dogs and can be introduced into the armamentarium of veterinary surgeons.

Key words: canine experiments; clinical implications; osteoplastic approach; spinal cord

INTRODUCTION

From 29th December 1882, when the outstanding Czech surgeon, Karel Maydl, performed the first successful intraspinal operation, consisting of the removal of spinous processes and vertebral arches (*laminae*) in a young carpenter suffering from complete paraplegia due to a compressive fracture of the vertebral column, this surgical method, addressed as *laminectomy* became a standard approach to different lesions localized in the spinal canal (10). Initially the method was introduced to human neurosurgery, subsequently to veterinary surgery, as well (1, 9, 11, 13, 14, 18). However, the removal of the *laminae* of vertebrae may result in serious disorders of the biomechanical properties of a body axis, the weakening of a backbone, and gradual development of a kyphotic deformity – predominatly in its cervical or thoracic parts (2). The risk of such a development is especially relevant in young individuals (2, 4, 17).

To avoid these unfavourable consequences of *laminectomy,* a new osteoplastic access to the spinal canal, addressed as *laminoplasty,* has been suggested in the for human neurosurgery (4, 5, 13, 17, 18, 20). We decided to test this surgical technique during experimental procedures in dogs.

MATERIALS AND METHODS

The experimental protocols were elaborated in compliance with the Animal Protection Act of the Slovak Republic No. 15/1995 and approved by the Regional State Veterinary Administration in Košice (decision No. SK P 53004) and by the Ethical Commission of the Neurobiological Institute of the Slovak Academy of Sciences in Košice.

The aim of the study was the direct measurement and continual registration of pO_2 in the spinal cord tissue during 30 minutes of a complete thoracic aorta occlusion just distal from a left subclavian artery origin (ischemic period) as well as during 30 minutes of re-established blood flow through

medullary vessels after removal of the tourniquet from the vessel (reperfusion period).

Nine adult mongrel dogs of both sexes, free from heart worm disease, weighing eighteen to twenty-five kilograms were used in the study. They were divided into two groups:

1. Sham controls (n = 3).
2. Ischemia-reperfusion injuries of the spinal cord (n = 6).

Before any attempt to use a new method in animals, technical aspects of laminoplasty were elaborated and repeatedly practiced on anatomical specimens of canine vertebrae (Figs. 1 and 2). Then the whole surgical procedure was applied in dogs in the first group. Animals were anaesthetized with pentobarbital (*Pentobarbitalum natricum* – „Pentobarbital" SPOFA, Praha) administered intravenously in a 30 $mg.kg^{-1}$ dose, in a supine position intubated with an endotracheal cannula („Portex", BERCK, Paris) of a diameter between 8—12 mm, and placed on a volume-cycled ventilator („Anemat N8", CHIRANA, Stará Turá). The anaesthesia was maintained further by a mixture of medical oxygen with 1—2 % of narcotan (*Halothanum thymolum stabilisatum* – „Narcotan", LÉČIVA, Praha).

Subsequently the dogs were put in a prone position, fixed in it, and after stabilisation of directly monitored blood pressure, arterial pO_2 and pCO_2 on normal canine levels, a cutaneous incision over spinous processes of three to five lumbar vertebrae was performed. Following the coagulation of bleeding vessels and cutting lumbodorsal fascia on both sides, paraspinal muscles were released bilaterally from posterior spinal bony structures (*processus spinosi, arcus vertebrae*) and held in an appropriate position by spreaders. Vertebral arches were divided bilaterally by a tooth-drill, excessive heat, produced by drilling, was absorbed by cool saline, constantly dripping on the active part of the instrument.

After the cutting of proximal and distal interspinal ligaments (*ligamenta interspinalia*), as well as yellow ligaments (*ligamenta flava, seu interarcualia*) bilaterally, it was possible to remove *laminae* as one piece and gain access to the dural sack containing the spinal cord (Fig. 3). Following durotomy (opening of *dura mater spinalis),* three dogs in the first group (sham controls) were maintained in general anaesthesia for thirty minutes, then the dural sack was sutured by an atraumatic needle with silk thread. The removed part of a vertebral column, stored during this period of experimental procedure in sterile saline with antibiotics (1 000 000 u.i. of *Benzylpenicillinum procainum,* BIOTIKA, Slovenská Ľupča and 1.0 g of *Streptomycin sulphate,* MEDEXPORT, Moscow) was put back and fixed in a correct position by suturing the interspinal ligaments at the cranial and caudal ends of laminotomy, and lumbosacral fascia to the spinous processes bilaterally.

Six animals of the second group were put and maintained in a general anaesthesia by using the same protocol as with the dogs of the first group. After gaining access to the dural sac containing spinal medulla (laminotomy), the position of the dog was changed from prone to the lateral on its right side and through a left-sided thoracotomy was loosely applied a tourniquet on the descending aorta (Fig. 4). Then the dog was put back on its abdomen, the *dura mater spinalis* was cut by scissors and the direct measurement of the oxygen tension in spinal cord tissue by a platinum electrode was started.

Following calibration of the registration apparatus, the blood flow in the aorta was interrupted by dragging the tourniquet (ischemic period). After thirty minutes of thoracic aorta occlusion, the tourniquet was removed (reperfusion period) and thoracotomic wound was sutured in anatomical layers. When the pO_2 values in spinal cord returned to pre-operative (initial) values (which in the majority of experimental animals took about fifteen minutes), the vertebral column of each dog was reconstructed using the same technique as in dogs of the first group.

Aseptic conditions were observed during all surgical procedures, tissues were moistened with a solution of penicillin and streptomycin in saline, lost fluids were replaced by intravenous infusions. After completing the operations, control as well as experimental animals were placed into separated, desinfected compartments, covered with warm blankets, and followed up to the point of complete recovery from anaesthesia. They were offered drinking water *ad libitum*, from the next day they received a full diet.

Seven dogs (two from the 1st plus five from the 2nd group) were killed under general anaesthesia by transcardial perfusion with 3,000 cc of saline and fixation by the same amount of 10 % neutral formaldehyde on the sixth postoperative days, while two (one from each group) were allowed to survive three more months.

RESULTS

Animals of both groups (experimental as well as sham controls) survived uneventfully rather complex surgical procedures. None of the dogs has shown any complication related to the laminoplasty or thoracotomy. All six animals of the second group had complete paraplegia of hind limbs resulting from ischemia and reperfusion damage of the spinal cord, which was the principal objective of the experiments, carried out on them. Three dogs serving as sham controls revealed no symptoms of neuronal damage. On the second post-operation day they walked, within ten days they even were able to run and jump. On plain roentgenograms of their spinal columns taken in two „classical" projections (antero-posterior and lateral), the site of the laminoplasty was almost unidentifiable (Fig. 5).

At the time of the dissections of the two dogs killed, following three months of survival, a firm extraspinal scar overlapping the completely healed laminoplasty was found. However, the scar tissue did not continue into the spinal canal. The originally interrupted *laminae* did not differ significantly from neighbouring vertebral arches – they were completely fixed in a very satisfactory position, fully ossified, the internal surface of the spinal canal was almost smooth (Fig. 6).

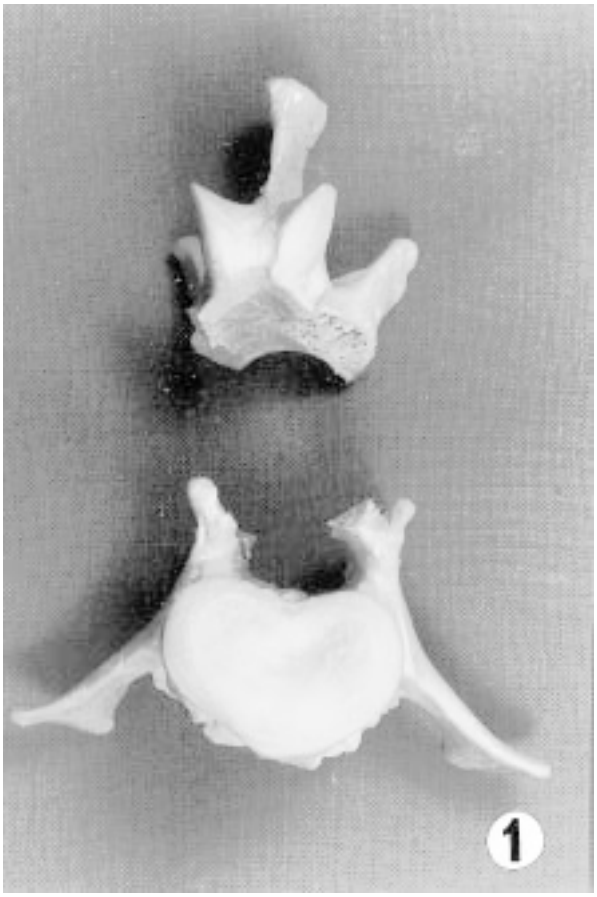

Fig. 1. Anatomical specimen of a dog vertebra following bilateral cutting of its arch by a tooth-drill

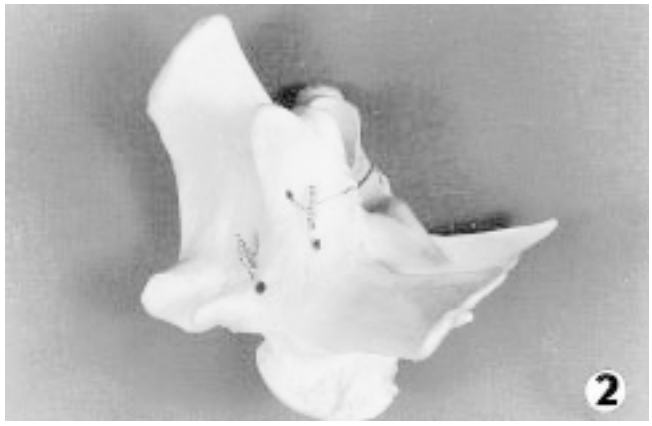

Fig. 2. Anatomical specimen of the same dog vertebra after laminoplasty. Vertebral arch fixed in a proper position with wire loops

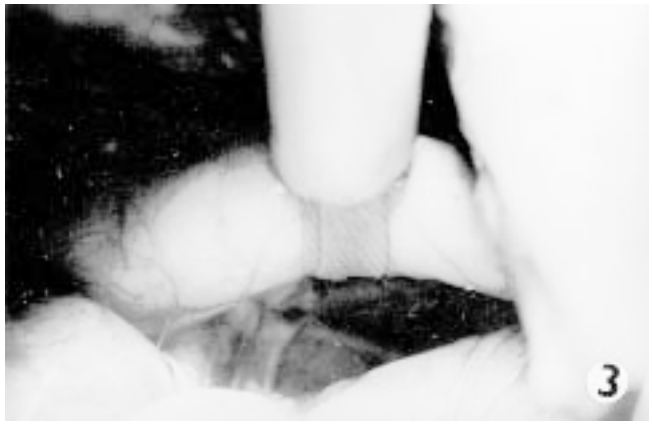

Fig. 3. A tourniquet applied on a thoracic aorta of a dog through the left-sided thoracotomy before constriction of the vessel

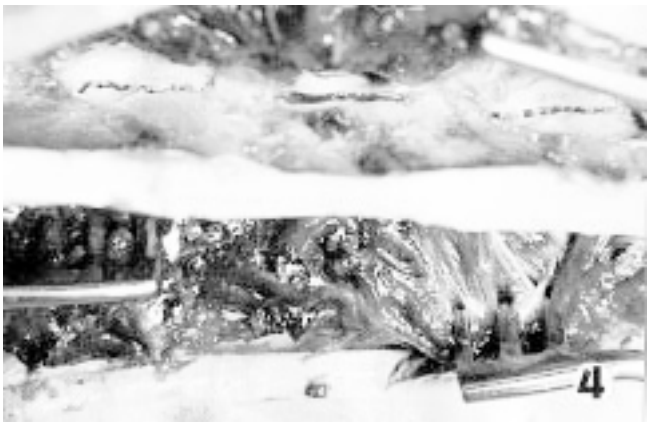

Fig. 4. The surgical situation in one of experimental animals. Separation of paraspinal muscles from spinous processes and vertebral arches of three lumbar vertebrae following laminotomy on the contralateral side

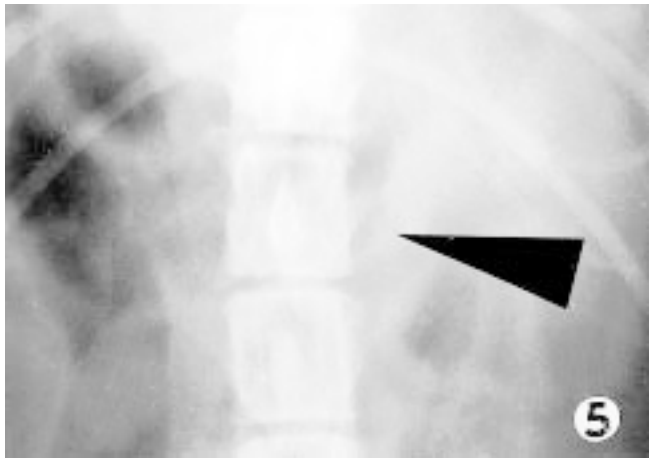

Fig. 5. Plain roentgenogram of a dog in an antero-posterior projection performed three months after laminoplasty of L_1 vertebra

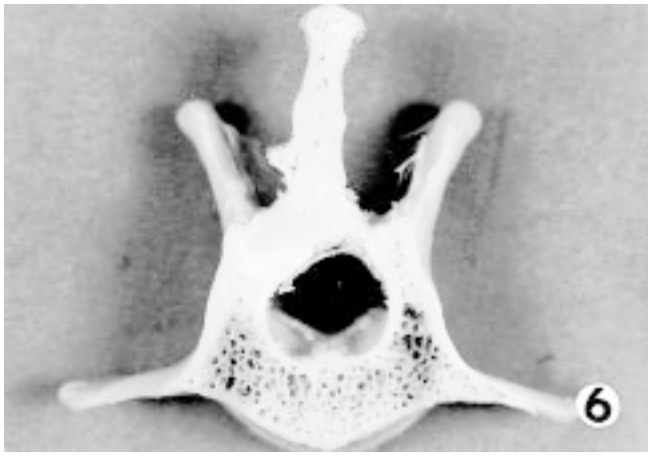

Fig. 6. Specimen of a canine vertebra satisfactorily healed during a three month's survival time after laminoplasty

DISCUSSION

Originally we intended to use smaller animals in experiments aimed at studying clinical and neurohistological manifestations of spinal cord damage induced by ischemia and reperfusion, as their utilisation is preferred nowadays (7, 19). However, the unavoidable consequence of a spinal cord lesion in rabbits is urinary retention, causing the early death of the animal. This fact, preventing the use of rabbits in experimental spinal cord injury models with a longer survival time, as well as serious problems with an endotracheal cannula insertion in both, rabbits and rats, make them unsuitable for studies necessitating a direct high thoracic aorta occlusion. That is why dogs or cats have been used in experimental neurosurgery and neurosciences quite frequently up to the present (1, 2).

Sufficiently large organs and the well-known physiological values in dogs made the endotracheal intubation, the maintenance of ventilation parameters, quite complicated surgical interventions, the postoperative care, the dosage of medicaments, as well as neurological and histopathological evaluations much easier (14—16). Moreover, since the operating room in our instituton is equipped with instruments and anaesthesiological apparatus designed for humans (and bigger animals), we decided to use dogs again in this present study.

The constant progress of pharmacology and development of new, potent anaesthesiological drugs has significantly influenced veterinary surgery in the last few years. Leading departments have begun to use combinations of *diazepamum, phenycyclidinum, cyclohexaminum* etc. in everyday veterinary clinical practice (6–8). We considered their utilisation in the planning phase of our experiments very seriously. Previous positive experience with a general anaesthesia induced by pentobarbital administered intravenously and maintained by a mixture of halothane with oxygen *via* endotracheal cannula connected to the anaesthesiological apparatus – securing long-term arteficial ventilation in a dog during procedures in an open thoracic cavity and spinal canal (which are considered complicated and highly demanding even in human surgery) (14—16) finally prevailed and we decided to use this technique repeatedly.

Following two studies of light-microscopic changes occurring in the spinal cords of dogs with paraplegia induced by ischemia and reperfusion, it becomes apparent that direct measurement and continual registration of intramedullary pO_2 could improve understanding of pathogenesis of this devastating complication of thoracoabdominal aortic operations (12, 15, 16).

Firstly, we intended to gain access to the spinal cord through a classical laminectomy; later on we decided to test a more up-to-dated approach into the spinal canal recommended in a contemporary neurosurgery – laminoplasty (2, 5, 13). Its principle is a bilateral interruption of the vertebral arches (laminotomy) and their reconstruction after completing the intraspinal procedure (5, 13, 17, 20).

The experimental operations shown that a laminotomy of one to three vertebrae in a dog is a quite straightforward procedure. Approaching the intraspinal structures (especially in lumbar area) is much simpler and less time consuming than by means of a classical laminectomy, which in dogs requires a considarable surgical experience, patience, and a wide range of bone instruments (Luer's, Kerrison's rongers) of different shapes and sizes. A certain disadvantage of utilisation of a tooth-drill or a high-speed-drill is the production of heat during the "cutting" of vertebral arches.

To prevent possible damage of neural structures in the bony vertebral canal of a dog, which is filled up to a virtually full extent by the dural sack and medulla, caudally to the level of the sixth lumbar segment, it is necessary to drip continually cool saline on the active part of the instrument during drilling (3, 9, 14, 17, 20).

The most favourable anatomical conditions for laminoplasty in dogs are in the lumbar part of their backbone. The situation is less suitable in the thoracic region, while the thick musculature and long spinous processes make this surgical approach to the cervical spinal canal almost impossible (3). That is why we would recommend a modification of the conventional laminectomy (instead of laminoplasty) for approaching a cervical intraspinal pathological process in these animals (3, 10, 14).

The majority of authors recommend for humans to fix the interrupted vertebral arches and spinous processes by titanium miniplates and screws or by wire loops during the final phase of laminoplasty (4). It is possible to proceed the same way with dogs, too. However, our experience has shown, that fixation of ligaments and lumbosacral fascia by interrupted silk sutures, granted a sufficiently strong retention of bony structures of the canine vertebral column. After completing the survival time, when the spinal cords were taken out for histological processing, the originally removed and subse-

quently reconstructed parts of vertebrae were found in a correct position.

Dissections of two animals killed three months following laminoplasty showed, that the method could be a very suitable technical improvement when a veterinary surgeon has to approach intraspinal pathological processes localised in the thoracic, lumbar or sacral parts of the canine vertebral canal.

CONCLUSIONS

The experience gained during the preparatory phase of experiments implemented on anatomical specimens of canine vertebrae, as well as the surgical procedures performed on nine adult mongrel dogs revealed significant advantages of laminoplasty in comparison with a conventional laminectomy. The new method grants in these animals sufficient access to the dural sack containing important neural structures, is easier to perform, has a lower complication rate and prevents the development of a post-laminectomic kyphosis.

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AN ASSESSMENT OF A PROPHYLACTIC DOSE OF ISOMETAMIDIUM CHLORIDE (SAMORIN®) ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS OF RABBITS EXPERIMENTALLY INFECTED WITH *Trypanosoma brucei brucei* (LAFIA STRAIN)

***Adedapo, A. A., Olayemi, F. O.**
Saba, A. B., Dina, O. A., Momoh, F. O.

Department of Veterinary Physiology and Pharmacology
University of Ibadan, Ibadan
Nigeria

adedapo3a@yahoo.co.uk

ABSTRACT

The effect of prophylactic doses of isometamidium chloride (Samorin®) on the haematological and serum biochemical parameters of rabbits experimentally infected with *Trypanosoma brucei brucei* (Lafia strain) was carried out in this study. Fifteen rabbits divided into three groups of five rabbits per group were used in this study. While group A served as control experiment, group B animals were infected with 0.5 ml of *T. brucei brucei* (8.9×10^5) intraperitoneally. Group C animals also received 0.5 ml of *T. brucei brucei* (8.9×10^5) intraperitoneally but were thereafter treated with isometamidium chloride after parasitaemia has been observed. The haematological parameters that were used in assessing this drug include: PCV, RBC, Hb, MCV, MCHC, MCH and WBC. For serum biochemistry, the parameters assessed were: Na^+, K^+, Cl^-, HCO_3^-, Ca^{2+}, PO_4^{2-}, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), total protein, albumin and globulin. Though these parameters experienced changes in their levels at the onset, but with the administration of this drug, all the parameters reverted to normality in their levels over time (21st day of drug administration). It thus showed that this drug has a chemotherapeutic effect on this strain of trypanosome, and is also capable of restoring haematological and biochemical parameters to their normal levels.

Key words: haematology; isometamidium chloride; rabbits; *T. brucei brucei*

INTRODUCTION

Trypanosoma congolense, T. vivax, T. simiae and *T. brucei* are among the major trypanosomes affecting livestock in Nigeria. The economic losses resulting from stunted growth, debility, poor reproductive performance or death in affected animals are unquantifiable – Agyemang (1), Jawara (21), Igbokwe (17), Otesile *et al.* (29).

The reality that African animal trypanosomiasis is the most important constraint to livestock production in the humid and sub-humid tropics and the fact that it affects about one-third of the African continent has led to the emergence of various control measures such as eradication of tsetse fly, chemoprophylaxis and chemotherapy with trypanocides such as homidium bromide, antrycide sulfate, diminazene aceturate, and isometamidium chloride in use – Atouguia (3), Onyeyili and Egwu (28), Schrevel *et al.* (33), Stevenson *et al.* (35). The one factor which however complicates the use of trypanocides more than any other in the enzootic area is the development of drug resistance in the trypanosome population – Geerts *et al.* (10), Gilman *et al.* (11).

* – Corresponding author: Dr. Adeolu A. Adedapo, Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan, Nigeria. Phone: 234 02 8102040, 08023928512, Fax: 234 02 810 1955. E-mail: adedapo3a@yahoo.co.uk

Another pharmacological problem in trypanosomiasis chemotherapy is the range of available drugs. Presently, only a few drugs are in use in animal trypanosomiasis in Africa and these include: homidium bromide (Ethidium®), pyrithidium bromide (Prothidium®), isometamidium chloride (Samorin®) and diminazene aceturate (Berenil®). Of these, only the last two are commonly available. Consequently, the range of available trypanocidal drugs in current use is limited. Apart from the side effects of these drugs, they are very expensive, and no new effective trypanocide has been introduced into the market in the last thirty years – Ilemobade (19), Onyia (27), Roderick *et al.* (31).

In endemic tsetse infested areas where cattle populations are reasonably stable, chemoprophylaxis is by far the best approach to the control of trypanosomiasis. Isometamidium chloride (Samorin®) is the drug of choice and in areas of constant heavy challenge; treatment may be required every six to eight weeks – Dina *et al.* (7), Jibike and Anika (21). Samorin® belongs to the phenanthridine chemical group and is used extensively as a chemoprophylactic drug at 0.5 to 2.0 $mg.kg^{-1}$ against *T. vivax, T. congolense* in cattle, sheep, goats and horses and *T. evansi* in donkeys and dogs. The chemoprophylactic effect lasts from three to six months depending on the severity of the challenge – Arowolo (2), Brander *et al.* (4).

This study, apart from assessing the efficacy of this drug also seeks to explore its curative effect knowing fully well that isometamidium chloride is a chemoprophylactic agent. This study also aimed at determining the resistance status of the Lafia strain of *Trypanosoma brucei brucei* to this drug.

MATERIALS AND METHODS

Experimental Animals

Ten cross bred weaners (rabbits – *Oryctolagus cuniculus*), males and females purchased from Post-rabbitry, Sango in Ibadan were used in this study. They were kept in clean metal cages in the experimental animal house of the Department of Veterinary Physiology and Pharmacology, University of Ibadan for a period of three months during which they were fed with grower's mash purchased from Bendel Feeds and Flour Mill as well as Ladokun Feeds Nigeria Limited. The rabbits had free access to clean water *ad libitum*. Four albino rats which served as reservoirs for the trypanosomes were also purchased from the Department of Veterinary Physiology and Pharmacology, University of Ibadan, Nigeria. They were also kept in clean metal cages in the experimental animal house of the Department of Veterinary Physiology and Pharmacology, University of Ibadan, and were fed with mouse cubes (Ladokun Feeds Nigeria Limited). They also had access to clean tap water *ad libitum*.

Trypanosome

Trypanosoma brucei brucei (Lafia strain) was obtained from the Department of Veterinary Pathology of the Faculty of Veterinary Medicine, University of Ibadan, where it has been passaged into laboratory rats and was inoculated into one of the four albino rats for storage for a period of ten to twelve days before passaging into another rat. The strain was originally obtained from the Nigerian Veterinary Research Institute (NVRI) Vom, near Jos, Nigeria and it was very virulent.

Drug

A sachet of isometamidium chloride (Samorin®) was used in the course of this study. It was a red powder, easily soluble in water. It was administered by deep intramuscular injection at the rate of $1mg.kg^{-1}$.

Experimental Design

The fifteen rabbits were divided into three groups of five rabbits per group. Before the experiments all the rabbits were bled so as to determine their weights, and basal haematological and biochemical values. Group 1 served as control and received normal saline. Group 2 rabbits were inoculated with 8.9×10^5 trypanosomes through the intraperitoneal route while Group 3 animals were inoculated with the same dose of trypanosomes. After parasitaemia has been detected in Group 3 rabbits, isometamidium chloride was administered to these animals.

Determination of Haematological Parameters

Blood samples were collected from each of the rabbits through the jugular vein into heparinised bottles for the determination of the haematological parameters. The red blood cell counts (RBC), packed all volume (PCV), haemoglobin (Hb) concentration, white blood cell (WBC) counts and the differential leukocytes count were as done by the standard procedure – Duncan *et al.* (8). From the values of the PCV, Hb and the RBC counts, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the Mean corpuscular haemoglobin concentration MCHC) were estimated. Leukocyte differential count was determined following Giemsa staining under a light microscope.

Determination of Serum Biochemical Parameters

The determination of sodium and potassium ions in the sera was performed using a flame photometer (Corning model 400, Corning Scientific Ltd; England) and the serum calcium level was measured by the cresolphthalein complexone technique – Toro and Ackerman (37). The serum phosphate level was determined using a photoelectric colorimeter (Gallenkamp and Sons Ltd.; England) – Gomori (12).

The serum total protein and albumin levels were determined by the Biuret method – Gornall *et al.* (13) while the globulin and globulin/albumin ratios were calculated accordingly to Coles (6). Serum urea and total bilirubin levels were determined using photoelectric colorimeter (Gallenkamp and Sons Ltd. England) – Coles (6). The cholesterol level on the other hand was determined by the direct method of Toro and Ackerman (37). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using a photoelectric colorimeter (Gallenkamp and Sons Ltd.; England) – Toro and Ackerman (37).

Statistical Analysis

The levels of significant differences between the mean values of the treated and control stages as well as pre-infection and post-infection phases were determined using the Student's *t*-test – Essex-Sorlie (9).

RESULTS

The effects of the trypanosomal infection on the rabbits were determined by studying the changes in their body weights for both the pre-infection and post-infection changes as well as changes in some of their haematological and serum biochemical parameters. For the body weight, the results showed that there was loss of body weight for all the infected rabbits when the pre-infection and post-infection periods were compared ($P < 0.05$) (Tables I – IV).

The results of this study with respect to parasitaemia also showed that there was an increase in the rate of multiplication of the trypanosomes in the rabbits. It thus showed that the organism was infective and virulent.

The results of this study with respect to changes in haematological parameters of rabbits showed that the levels of PCV, Hb, and RBC of infected rabbits experienced significant reduction ($P < 0.05$) when compared with the control and the pre-infection periods. The RBC indices such as MCV, MCH, also experienced significant changes. WBC also experienced a significant increase in this study (Tables I and II).

Table I. Pre-infection haematological parameters of rabbits and body weights

PARAMETERS	Group 1 (Control)	Group 2	Group 3
PCV (%)	32.6 ± 2.6	33 ± 2.5	33.0 ± 2.6
Hb ($g.dl^{-1}$)	11.1 ± 3.4	14.0 ± 2.3	19.9 ± 1.4
RBC ($10^6.\mu l^{-1}$)	4.1 ± 1.2	4.1 ± 1.8	2.9 ± 0.1
MCV (fl)	86.7 ± 22.9	85.0 ± 20.8	112.0 ± 6.6
MCHC (%)	33.4 ± 10.6	42.2 ± 3.8	60.3 ± 2.7
MCH (pg)	71.7 ± 26.2	35.6 ± 7.1	67.4 ± 3.3
WBC ($10^3.\mu l^{-1}$)	8.7 ± 0.7	7.9 ± 0.8	6.4 ± 0.8
Body weight (kg)	1.4 ± 0	1.3 ± 2.1	1.3 ± 2.6

Table II. Effects of *T. brucei brucei* haematological parameters and on the body weights of rabbits (Post-infection)

PARAMETERS	Group 1 (Control)	Group 2	Group 3
PCV (%)	37.3 ± 5.1	15.7 ± 2.6^{ab}	40.0 ± 4.3^{ab}
Hb ($g.dl^{-1}$)	20.6 ± 1.9	6.3 ± 0.7^{ab}	20.7 ± 0.7^{a}
RBC ($10^6.\mu l^{-1}$)	3.4 ± 1.0	1.2 ± 0.2^{ab}	4.2 ± 0.2^{ab}
MCV (fl)	114.7 ± 30.9	131.8 ± 37.0^{ab}	114.0 ± 25.4^{ab}
MCH (pg)	63.6 ± 18.7	51.4 ± 4.2^{ab}	50.0 ± 1.7^{b}
MCHC (%)	55.5 ± 6.6	42.6 ± 12.7	53.5 ± 5.7^{a}
WBC ($10^3.\mu l^{-1}$)	6.6 ± 0.8	6.5 ± 0.8^{a}	53.6 ± 0.4^{a}
Body weight (kg)	1.5 ± 0	0.97 ± 1.7^{ab}	1.2 ± 2.3^{ab}

a — significant values at $P < 0.05$ (pre-infection and postinfection phases)

b — significant values at $P < 0.05$ (control i.e. group 1 and the experimental groups)

Administration of isometamidium chloride to Group 3 cleared the parasitaemia earlier observed and then restored the haematological and serum biochemical parameters to their normal levels at about the 21st day after drug administration (Table II).

The results of this study showed that *T. brucei brucei* caused significant changes on some serum biochemical parameters of rabbits. For instance Na^+, HCO_3^- Cl^-, total protein and globulin all experienced significant reduc-

Table III. Pre-infection serum biochemical parameters and body weight of rabbits

PARAMETERS	Group 1 (Control)	Group 2	Group 3
Na^+ ($mmol.l^{-1}$)	142.2 ± 3.4	134.0 ± 5.4	135.0 ± 3.3
K^+ ($mmol.l^{-1}$)	4.1 ± 0.4	4.2 ± 0.2	5.1 ± 0.7
Ca^{2+} ($mmol.l^{-1}$)	8.5 ± 0.1	8.6 ± 0.1	7.6 ± 0.2
$P0_4^{2-}$ ($mmol.l^{-1}$)	4.5 ± 0.1	4.4 ± 0.2	4.3 ± 0.3
Cl^- ($mmol.l^{-1}$)	105.6 ± 1.4	102.0 ± 1.4	102.3 ± 1.2
HCO_3^- ($mmol.l^{-1}$)	23.6 ± 0.8	22.7 ± 2.1	22.4 ± 0.4
Urea ($mmol.l^{-1}$)	13.0 ± 3.1	15.0 ± 2.5	11.0 ± 2.3
Creatinine ($\mu mol.\ l^{-1}$)	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.2
Total Protein ($g.l^{-1}$)	4.6 ± 0.4	4.3 ± 0.1	4.5 ± 0.3
Albumin ($g.l^{-1}$)	2.1 ± 0.1	2.0 ± 0	2.2 ± 0.2
Globulin ($g.l^{-1}$)	2.5 ± 0.3	2.3 ± 0.1	2.3 ± 0.3
A/G Ratio	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.2
AST (IU)	24.8 ± 2.7	19.3 ± 3.1	24.3 ± 3.1
ALT (IU)	16.8 ± 3.3	15.3 ± 3.8	15.9 ± 2.3
ALP (IU)	106.2 ± 8.9	110.3 ± 7.9	107.4 ± 6.2
Body weights (kg)	1.4 ± 0	1.3 ± 2.1	1.3 ± 2.6

Table IV. Effects of *T. brucei brucei* on the serum biochemical parameters of the body weight of rabbits (Post-infection)

PARAMETERS	Group 1 (Control)	Group 2	Group 3
Na^+ ($mmol.l^{-1}$)	139.2 ± 1.4	127.3 ± 5.7^{b}	130.0 ± 10.0^{b}
K^+ ($mmol.l^{-1}$)	4.0 ± 0.7	5.0 ± 1.0^{a}	7.1 ± 2.3^{b}
Ca^{2+} ($mmol.l^{-1}$)	8.4 ± 0.1	8.3 ± 0.1^{a}	8.5 ± 0.2^{a}
$P0_4^{2-}$ ($mmol.l^{-1}$)	4.6 ± 0.1	4.8 ± 0.3^{a}	4.4 ± 0.1^{a}
Cl^- ($mmol.l^{-1}$)	104.3 ± 1.9	100.0 ± 1.6^{ab}	99.7 ± 6.9^{b}
HCO_3^- ($mmol.l^{-1}$)	25.0 ± 1.0	20.7 ± 0.9^{ab}	22.0 ± 1.6^{b}
Urea ($mmol.l^{-1}$)	13.3 ± 4.7	14.0 ± 2.8	11.3 ± 0.9
Creatinine ($\mu mol.\ l^{-1}$)	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
Total Protein ($g.l^{-1}$)	4.6 ± 0.4	4.3 ± 0.1	5.9 ± 0.9^{ab}
Albumin ($g.l^{-1}$)	2.1 ± 0.1	2.0 ± 0	2.0 ± 0^{a}
Globulin ($g.l^{-1}$)	2.5 ± 0.3	2.3 ± 0.1^{b}	2.3 ± 0.1^{b}
A/G Ratio	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
AST (IU)	17.5 ± 3.2	28.0 ± 5.9^{ab}	23.3 ± 3.4^{b}
ALT (IU)	12.3 ± 2.1	15.3 ± 4.1	15.0 ± 0.8
ALP (IU)	81.3 ± 4.3	114.3 ± 8.7^{b}	110.7 ± 9.0^{b}
Body weights (kg)	1.5 ± 0	0.97 ± 1.7^{ab}	1.2 ± 2.3^{ab}

a — significant values at $P < 0.05$ (pre-infection and post-infection phases)

b — significant values at $P < 0.05$ (control i.e. group 1 and the experimental groups)

tion in their levels when pre-infection and post-infection periods of Group 2 were compared with the controls. The A/G ratio however experienced a significant increase for all the experimental groups. AST, ALT and ALP all experienced a significant increase in their levels (Table IV).

DISCUSSION

Trypanosomes are known to cause trypanosomiasis in cattle, sheep, goat, horse, and other mammals including man. In this study, there is no doubt that *T. brucei brucei* caused an infection in rabbits. This is because the peripheral blood examinations revealed a decrease in the erythrocyte count, haemoglobin concentration, and packed cell volume. This is consistent with *T. brucei brucei* infection because it was said that the most characteristic symptom of this trypanosome is anaemia and it results from the inhibition of the formation of red blood cells in the haemopoietic tissues as well as the destruction of those red cells already in circulation – Hunter and Luckins (15), Igbokwe (16), Igbokwe *et al.* (18), Jones *et al.* (23), Ogunsanmi *et al.* (26). The implication of this is that the reduction of these parameters may lead to anaemia, which may be functionally defined as the decreased oxygen-carrying capacity of the blood.

The easiest and most accurate laboratory indication of anaemia is reduction of the PCV or haematocrit below the normal range for the species – Morris (24). There are three pathophysiologic mechanisms for development of anaemia: blood loss, increased erythrocyte destruction (haemolysis) and inadequate erythrocyte production. The red blood cell (RBC) indices are used to characterize and classify anaemias – Straus (36). From this study, MCV, MCH and MCHC all experienced significant changes in their values.

It has also been shown that animals infected with *T. brucei brucei* are immunosuppressed and frequently have intercurrent bacterial, viral or other parasitic infections that may mask or complicate the basic clinical syndrome – Pearson *et al.* (30), Rurangirwa *et al.* (32), Seifert (34), Whitelaw *et al.* (38). In this study, the white blood cells also experienced significant decrease in their levels. It thus shows that the immunosuppressive attributes of this organism may involve cell-mediated immunity. This is because the blood monocytes and tissue macrophages constitute the mononuclear phagocyte system known as the reticuloendothetial system – Duncan *et al.* (8), Jain (19).

The functions of tissue macrophages include sustained phagocytic activity to remove dead and damaged tissue; microbicidal action against some bacteria, viruses, fungi, and protozoa regulation of immune response in both afferent and efferent limbs; tumour defense; regulation of haematopoiesis; tissue repair and remodeling; and secretion of monokines, lysosomal enzymes and other substance such as coagulation factors that have wide-resulting biologic importance. It should be noted that monocytes are produced in the bone marrow and differentiate into either myeloblasts or monoblasts. Monocytes once released into blood transform into macrophages once they enter into body cavities and tissues – Morris (25).

This study revealed that inoculation of *T. brucei brucei* caused significant changes in the levels of the serum biochemical parameters such as Na^+, K^+, Ca^{2+}, PO_4^{2-}, Cl^-, and HCO_3^- (Tables III and IV). Changes in water balance are responsible primarily for changes in serum sodium concentration.

Hyponatraemia is an indication of a relative water excess, whereas hypernatraemia is an indication of a relative water deficit – Carlson (5). It may be safe to say that hyponatraemia occurred in this study. Hyponatraemia is often but not invariably associated with conditions that cause sodium depletion such as vomiting, diarrhoea, excessive sweat losses, and adrenal insufficiency – Carlson (5).

Serum potassium concentration in this study showed a significant increase, which may suggest that hyperkalaemia exists in this work. Hyperkalaemia may result from a redistribution of potassium from the intracellular fluid (ICF) into the extracellular fluid (ECF) space. It is most commonly seen with altered intake and absorption – Carlson (5). One may be tempted to think that hyponatraemia and hyperkalaemia noticed in this study are due to distribution of these cations between the ECF and ICF compartments.

The biochemical changes and thence the pathogenic effect of this organism noted in this study could be attributed to the fact that: (a) metabolic effects of the *Trypanosoma*, which withdraw essential nutrients and produce toxic metabolites. The consumption of glucose, production of pyruvate and deaminization of the amino acids tyrosine and Tryptophan seem to be especially important, therefore intermediate metabolic disorders are the result; (b) the actions of the secretions, such as acid phosphatase which activate the complement system, and pharmacokinetic active substances like serotonin and kinine also have direct pathogenic effect – Seifert (34). The significant reduction in the weight of the experimental animals in this study could therefore be attributed to these biochemical disorders as already explained.

Trypanosoma brucei brucei infection in cattle is usually characterized by a mild and almost symptomless course but in dogs, the infection with *Trypanosoma brucei brucei* will lead to death after one to three weeks – Holmes (14), Seifert (34).

This study has shown that isometamidium chloride (Samorin®) has curative effect on trypanosomosis caused by *Trypanosoma brucei brucei*. It thus confirmed the fact that this drug has both a curative and chemoprophylactic effect. This study also showed that isometamidium chloride (Samorin®) is an effective trypanocide even in rabbits since the administration of this drug restored all the haematological and biochemical parameters to their pre-infection level 21 days after its administration.

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THE RADIOPROTECTION OF MICE BY THE BACTERIAL EXTRACT BRONCHO-VAXOM AND INHIBITOR OF PROSTAGLANDIN PRODUCTION — INDOMETHACIN AND THEIR COMBINATION ON HISTONES IN THE MOUSE LIVER

Kožurková, M., Fedoročko, P., Mišúrová, E.

Institute of Biological and Ecological Sciences
P. J. Šafárik University, Moyzesova 11, 040 57 Košice
The Slovak Republic

kozurkova@kosice.upjs.sk

ABSTRACT

The effect of the bacterial extract Broncho-Vaxom (radioprotective immunomodulator, 500 mg/mouse i.p. – 24 hours before radiation) and indomethacin (inhibitor of prostaglandin production, 40 mg/mouse i.m. – 24 hours and 3 hours before irradiation) on the radiation-induced changes in histones in mice was investigated. Both agents were administered either alone or in combination. Administration of Broncho-Vaxom alone 24 hours before irradiation caused a slightly quicker increase in both the concentration and content of histones in the period of radiation damage recovery. A similar effect was found after administration of indomethacin alone. The combined modality treatment Broncho-Vaxom and indomethacin was more effective than single agent treatments. The concentration and content of murine histones was increased in all investigated time intervals. The relative proportions of some histone fractions and histone variant H1 were temporarily altered in mice treated with Broncho-Vaxom and/or indomethacin.

Key words: Broncho-Vaxom; histones; indomethacin; mice liver; radioprotection

INTRODUCTION

Histones play an essential role in the structure and function of all eukaryotic cells. In nucleosomes, about two turns of DNA are wound around an octamer of histone proteins (two each of H3, H4, H2A, H2B). Molecules are interconnected from the outside by histone H1 (16). Histone H1 acts as a general repressor at one level of the regulation of transcription in eukaryotes by organizing nucleosomes into condensed forms of chromatin, thereby making the DNA inaccessible to transcription machinery (3, 5, 8, 15).

H1° is one of the histone variants of the H1 family. Like other variants of the H1 family, H1° is located on the linker region of nucleosomes. As was previously reported (20) presented H1° leads to an increase in the resistance of chromatin to micrococcal nuclease digestion. Results of this kind suggest that H1° could be associated with or responsible for a more compact conformation of chromatin.

Ionizing irradiation causes profound changes in histones and nucleic acids in quiescent or proliferating liver cells (12, 13). Radiation changes can be prevented by the administration of some chemical compounds. It has been found that many agents, which are capable of non-specific stimulation of immunological and haemopoietic responses can also function as radioprotectants (6, 21).

Indomethacin (INDO) is a nonsteroid anti-inflammatory drug. Inhibiting the synthesis of prostaglandins, which play a role in haemopoietic stem cell proliferation, it interferes with cell metabolism and *in vivo* proliferation of lymphocytes and increases the rate of blastogenesis of splenocytes activated by lectins. On the other hand, an antiproliferative effect of indomethacin at higher doses has also been reported.

The antiproliferative effect underlines its antitumor activity. The response to indomethacin treatment depends mainly on whether or not the tumors produce prostaglandins. The therapeutical potential of indomethacin is likely to lie only in its combination with other treatments.

Broncho-Vaxom (BV), lyophilized fractions of bacterial extract (endotoxin free) from 8 strains is used as a polyvalent immunotherapeutic agent in the treatment of respiratory tract infections, particularly acute and chronic bronchitis (17). The non-specific immunostimulant properties of BV have been demonstrated by several investigators (1, 4, 11). In this connection, it may be of importance, that the incubation of the macrophage cell line with BV has been reported to induce interleukin 1 (IL-1) secretion and the production of prostaglandins (2).

Fedoročko *et al.* (6, 7) have found that the administration of the bacterial extract BV in combination with indomethacin was more effective, than single agent treatments in accelerating cellularity and GM-CFC regeneration in mouse bone marrow, but not in the spleen. Combined administration of BV and INDO to mice prior to lethal irradiation exerted an additional radioprotective effect and protected 95 % of the C57B1/6 mice.

In the present study we have tried to determine whether BV or INDO alone or in combination modified the radiation-induced changes in the concentration and content of histones and relative proportion of histone fractions and subfraction $H1^0$ in the liver of mice and thus to contribute to the understanding of the mechanisms of action of these agents.

MATERIAL AND METHODS

Female C57B1/6 mice eight to ten weeks old were obtained from Velaz (Prague, Czech Republic). The animals were guaranteed for a period of two weeks. They were housed in rodent cages, five to seven animals per cage at about 22 °C and were given Velaz Altromin 1320 St laboratory chow and tap water acidified to pH 2.4 *ad libitum*.

Research was conducted according to the principles enunciated in the *"Guide for Care and Use of Laboratory Animals"*, prepared by the State Veterinary Office of the Slovak Republic Bratislava.

Broncho-Vaxom – BV (Biogal Pharmaceutical Works, Debrecen, Hungary, under license from OM Laboratories, Geneva, Switzerland) is a lyophilized extract of the most common bacteria of upper respiratory tract and free of endotoxins (less than 0.0002 % by *Limulus* and pyrogenicity test) (2). Immediately before use, the drug was resuspended in saline at a volume of 0.4 ml and administered intraperitoneally 24 hours before irradiation or decapitation (in the case of nonirradiated mice) at a dose of 500 mg per mouse. This time interval has been shown to be an effective administration schedule for most of the immunomodulators including BV (4). Control animals received i.p. saline in the same volume and at the same time as the treated animals.

Indomethacin treatment (INDO, Sigma Chemical Co., St. Louis, USA) was prepared by dissolving 10 mg in one ml of 95 % ethyl alcohol. This solution was then diluted to a working concentration with Dubelcco's phosphate-buffered saline (TechGen Int. LTD, UK) and injected i.m. at 40 mg per mouse at a volume of 0.2 ml 24 h and 3 hours before irradiation or decapitation (in the case of non-irradiated mice). Both drugs were administered either alone or in combination.

Mice were placed in plexiglas containers and whole-body (unilaterally) exposed to gamma radiation with the dose of 7.0 Gy (at a dose rate of 0.4 Gy/min), 24 hours after the first injection. A Chisostat ^{60}Co source (Chirana, Czech Republic) was used for all irradiation.

Isolation of liver nuclei was carried out according to the method of Grunicke *et al.* (9).

Protein concentration was determined by Lowry method (14) with bovine serum albumin as a standard. Electrophoresis was carried out using the method by Panyim and Chalkley (18).

The values given in the figures and tables represent the means ± the standard errors (S.E.M.). The statistical significance of the differences was evaluated using Peritz' F-test (10).

RESULTS

Concentrations and content of histones. In the liver of non-irradiated rats, BV administered alone caused an increase in the concentration and content of histones at the beginning of the experiment (24 hours after administration). Administration of INDO alone or in combination with BV (BV and INDO) caused no significant

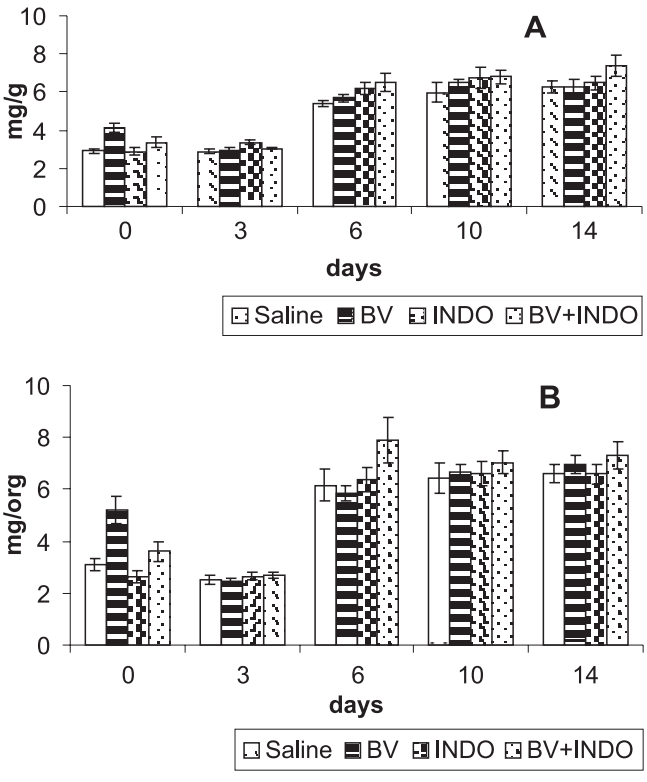

Fig. 1. Concentration (A) and content (B) of histones in the liver of mice irradiated by gamma rays (7 Gy) after application of saline (irradiated controls, C) or treatments with Broncho-Vaxom (500 mg/mouse, BV) and/or indomethacin (2 × 40 mg/mouse, INDO)

^{x} — $P \leq 0.05$, ^{xx} — $P \leq 0.001$ for differences between non-irradiated (0 day) and irradiated mice

^{o} — $P \leq 0.05$, ^{oo} — $P \leq 0.001$ for differences between non-treated (saline) and treated animals (BV, INDO, BV + INDO)

changes in concentration and content of histones compared with control (Figs. 1 A, B).

In animals irradiated with a dose of 7.0 Gy the values of histone concentration and content were slightly decreased on the third day after irradiation. On the following days, restoration took place and on the sixth day a significant increase was found which lasted till the fourteenth day after irradiation.

Administration of BV alone 24 hours before irradiation caused a slightly quicker increase in both the concentration and content of histones in the period of restoration. A similar sequence was found after a single administration of INDO alone.

The combined modality treatment (BV and INDO) was more effective than single agent treatments not only in the course of restoration, but also in the course of the rise of radiation damage. The concentration and content of murine liver histones were increased for all time interval, mainly from the sixth to the fourteenth day after irradiation.

Relative proportion of histone fractions. As shown in Table I the relative proportion of major histone fractions appeared to be quite constant in non-protected animals.

After application of BV or INDO alone some significant changes in the main histone fractions were found.

The combination of BV and INDO caused an increase in the histone fraction H1 to the detriment of histone fractions H2 + H3 on the sixth day after irradiation. The opposite sequence was found on the tenth and fourteenth days after irradiation.

Histone variant H1°. Histone variant, though a very small part of the total histones, presented about one fifth of the H1 histone fraction in the control animals (Table II).

After irradiation the H1° proportion only changed slightly.

The administration of BV or INDO alone caused no changes in non-irradiated mice. The combination of these two agents, however, caused rapid significant increase (from 26 to 40 %) on the 24 hours after application.

Pretreatment of irradiated mice with BV and/or INDO caused a transient increase of the values of this subfraction on the third day, and repeatedly on the tenth and fourteenth days.

DISCUSSION

Broncho-Vaxom is one of the radioprotective immunomodulators that, like polysacharides and interleukin-1 (19) among other effects, enhances the radioprotective effects of thiol compounds.

The exact mechanism of Broncho-Vaxom action is not fully understood, but experimental studies have indicated that BV enhances immune response (4) including the activity of peritoneal macrophages (1). Accessory cells such as macrophages, fibroblasts and monocytes participate in immune responses in a number of ways, including the production of bioactive agents.

The liver is the largest organ in the body providing a large number of essential functions for the organism. It is the center for the metabolism of nutrients and drugs and plays a key role in the non-specific immune system by harboring Kupffer cells, the majority of all macrophages. The liver is the main site for the synthesis of many different metabolites and releases most of the newly synthesized proteins. All these functions of the liver must be coordinated and regulated in response to metabolic changes and minor or major injuries. This is ac-

Table I. Relative proportion of histone fractions (%) in the liver of mice irradiated by gamma rays (7 Gy) after application of saline (irradiated controls, C) or treatment with Broncho-Vaxom (500 mg/mouse, BV) and/or indomethacin (2 × 40 mg/mouse, INDO)

DAYS/ TREAT-MENTS	C			BV			INDO			BV + INDO		
	H1	H2 + H3	H4	H1	H2 + H3	H4	H1	H2 + H3	H4	H1	H2 + H3	H4
0	20.12 ± 1.89	62.52 ± 1.32	17.35 ± 1.70	21.72 ± 1.37	57.89^{x} ± 0.95	20.38 ± 1.30	23.27^{x} ± 1.41	42.00^{xx} ± 2.30	14.71 ± 1.14	21.45 ± 2.48	58.74^{x} ± 0.78	19.79 ± 0.80
3	21.81 ± 2.73	59.06 ± 0.76	19.13 ± 1.78	22.85 ± 3.66	57.81 ± 0.65	19.32 ± 1.75	25.50 ± 3.9 0	60.06^{oo} ± 0.80	14.43 ± 1.94	18.65 ± 5.52	62.21 ± 3.00	19.09 ± 0.41
6	20.01 ± 1.84	64.43 ± 2.39	15.54 ± 2.50	19.21 ± 2.06	59.88 ± 0.96	20.90 ± 1.80	21.86 ± 2.53	62.61^{oo} ± 2.70	15.29 ± 3.02	30.12^{xx} ± 2.75	54.57^{xx} ± 2.57	15.29 ± 3.02
10	23.44 ± 2.11	60.90 ± 1.84	12.24 ± 2.16	24.88^{x} ± 2.10	53.17^{xx} ± 1.21	15.43 ± 2.16	22.51 ± 2.26	61.69^{oo} ± 2.17	15.79 ± 2.86	17.60^{x} ± 1.53	66.63^{oo} ± 1.48	15.76 ± 1.8
14	20.83 ± 3.1	61.94 ± 2.93	17.28 ± 4.23	20.12 ± 3.74	63.00 ± 1.65	16.87 ± 1.78	22.10 ± 1.45	58.65 ± 1.20	19.23 ± 1.28	18.45 ± 1.23	63.40 ± 0.97	18.13 ± 1.21

x — $P \leq 0.05$, xx — $P \leq 0.001$ for differences between non-irradiated (0 day) and irradiated mice
o — $P \leq 0.05$, oo — $P \leq 0.001$ for differences between non-treated (saline) and treated animals (BV, INDO, BV + INDO)

Table II. The percentage of H1° variant within the H1 histone fraction in the liver of mice, irradiated by gamma rays (7 Gy) after application of saline (irradiated controls, C) or treatment with Broncho-Vaxom (500 mg/mouse, BV) and/or indomethacin (2 × 40 mg/mouse, INDO)

DAYS/ TREAT-MENTS	C	BV	INDO	BV + INDO
0	25.78 ± 2.24	22.87 ± 1.36	21.01 ± 3.78	39.84 ± 4.25
3	25.90 ± 2.13	29.43 ± 1.60	26.96 ± 1.82	29.25 ± 2.46
6	23.08 ± 2.69	23.60 ± 1.42	24.18 ± 1.34	20.64 ± 0.55
10	21.13 ± 2.03	21.28 2 ± 2.80	9.13 ± 2.12	22.53 ± 1.78
14	27.81 ± 2.27	30.35 ± 2.01	31.61 ± 1.66	19.82 ± 1.66

^{x} — $P \leq 0.05$, ^{xx} — $P \leq 0.001$ for differences between non irradiated (0 day) and irradiated mice
^{o} — $P \leq 0.05$, ^{oo} — $P \leq 0.001$ for differences between non-treated (saline) and treated animals (BV, INDO, BV + INDO)

complished by metabolites, the autonomous nerve system, the endocrine system and by cytokines, which form a complex network of mediator molecules. Cytokines modulate liver metabolism in many ways. Synthesis of many proteins such as acute phase proteins is regulated by cytokines such as IL-1, IL-6, IL-11, TNF, leukemia inhibitory factor, transforming growth factor, epidermal growth factor, etc.

Cytokines play an important role in the pathogenesis of liver diseases and liver fibrosis, which is the common morphological reaction after chronic injury of the liver. The uncontrolled production of some cellular and extracellular matrix proteins and their impaired degradation destroy the architecture of the liver and its function.

Radiation injury to tissues is a common sequel to radiotherapy. Especially, the radiotoxic effect on the hematopoietic cell system as well as on parenchymal tissues, like lung, kidney and liver, is the dose-limiting factor in radiotherapy (21).

In the study described in this paper, we evaluated the radioprotective ability of BV (haemopoietic stimulant) and INDO (inhibitor of prostaglandin production), two agents that individually can influence liver histones in irradiated mice and enhance their effect when administered in combination.

The effect of BV is presumed to be mediated through its ability to induce the production of the endogenous haemopoietic growth factor production from predominantly radioresistant macrophage cell (Kupffer cells) populations. For example, both interleukin-1 (IL-1) and prostaglandin E_2 (PGE_2) production (1) have been demonstrated following *in vitro* BV administration, which may indicate that BV administration can accelerate the restoration of functional haemopoietic cells *via* production of cytokines that stimulate a broad spectrum of progenitor cells. Cytokine production is modulated by indomethacin, an inhibitor of prostaglandin (PG) synthesis.

According to Fedoročko *et al.* (6, 7), the radioprotective effects of the immunomodulator and inhibitor of prostaglandin production combination could be explained by the additive action of both drugs.

Our results showed the beneficial effect of BV on the development and recovery of radiation-induced changes of histones in mice livers. Application of BV stimulated the augmentation in histones in the livers of mice irradiated with a dose of 7 Gy on the sixth day until the fourteenth day after irradiation. Also indomethacin alone had similar effect. But the combined administration of BV and INDO to mice exerted an additional radioprotective effect.

Protecting normal tissues from the damaging effects of ionizing radiation would have important impact on the development of new strategies in radiotherapy. Experiments analyzing the radioprotective potential of various cytokines has increased our knowledge of the mechanisms by which cytokines can protect and enhance normal tissue recovery from radiation injury. Some cytokines appear to be implicated in an early step in the regulated initiation of the repair of potential lethal damage in cells and they are important inducers of oxygen radical scavengers.

IL-1, IL-12 and TNF2 represent the most prominent radioprotective cytokines. The specific molecular mechanisms by which radioprotective cytokines, like IL-1 and IL-12 repair the radiation damage to tissues still remain to be elucidated. Based on cell biological data it can be postulated that autocrine/paracrine regulated tissue repair processes involving multicellular interactions exist and that cytokines like IL-1/IL-12 play an important role in initiating and promoting such tissue "repair loops". In principle, the radioprotective and potentially therapeutic effects of supplementary pharmacological doses of cytokines may be exerted by amplifying levels of endogenous, radiation-induced cytokines that induce repair processes (2, 21).

ACKNOWLEDGEMENTS

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CHANGES IN THE SPINAL TRIGEMINAL TRACT AND ITS NUCLEUS INDUCED BY SPINAL CORD ISCHEMIA-REPERFUSION INJURY IN DOGS

Šulla, I.[1], Vanický, I.[2], Balik, V.[3]

[1] University of Veterinary Medicine, Komenského 73, 041 81 Košice
[2] Neurobiological Institute of the Slovak Academy of Sciences
Šoltésovej 4, 040 01 Košice
[3] Department of Neurosurgery, P. J. Šafárik University School of Medicine
Tr. SNP 1, 040 01 Košice
The Slovak Republic

sulla@uvm.sk

ABSTRACT

In a group of nine adult mongrel dogs of both sexes weighing between 18 and 25 kilograms the authors have studied the possible connection between spinal cord and specific brain stem structures. In three animals (sham controls) they performed a left-sided thoracotomy only. In the six experimental animals they occluded the thoracic aorta by a tourniquet, approached through a left-sided thoracotomy, just distal to the origin of the left subclavian artery for thirty minutes. Procedures in control and experimental animals were performed with a general anaesthesia induced by pentobarbital (30 mg.kg^{-1} i.v.) and maintained with a mixture of 1—2 % halothane with oxygen *via* an endotracheal cannula. On the sixth post-operative day all the dogs were killed by deep general anaesthesia (pentobarbital i.v. in a dose of 50 mg.kg^{-1}) with a perfusion (3000 ml of saline) and fixation (the same volume of 10 % neutral formaldehyde). Thirty µm thick sections from spinal cord and brain stem were cut by a microtome and processed according to the Nauta staining method. Light-microscopic observations showed distinct changes in the spinal trigeminal tract, its nuclei and some ascending spinal systems originating in the lumbosacral and lower thoracic spinal cord segments. These projections are very probably related to the posterior spinocerebellar tract.

Key words: canine experimental model; ischemia-reperfusion paraplegia; trigeminal complex changes

INTRODUCTION

Excruciating pain, which is the main symptom of primary trigeminal neuralgia and frequent relapses of the disease in spite of the application of the most sophisticated treatment procedures, has focused the interest of neuroanatomists, neuropathologists, neurologists and neurosurgeons on this cranial nerve, its nuclei and surrounding structures (3–6, 13, 14, 17). Questions dealing with the localisation and origin of its primary afferent fibres were studied in detail (9, 11, 14). It has been almost automatically supposed that axons of pseudounipolar cells of the semilunar ganglion (Gasser's ganglion) are the only source of afferent impulses coming to the spinal trigeminal tract, its nuclei and, subsequently, to the thalamus and cortical structures (6, 9, 13, 14).

Light-microscopic observations of changes developing in the spinal trigeminal tract simultaneously with spinal cord neuronal damage induced by ischemia and reperfusion in a canine experimental model of paraplegia, imply the existence of spinal projections to structures of the trigeminal complex. This important detail inspired the authors to pay more attention to the problem.

MATERIAL AND METHODS

The experimental protocols were elaborated in compliance with the Animal Protection Act of the Slovak Republic No. 15/1995 and approved by the Regional State Veterinary

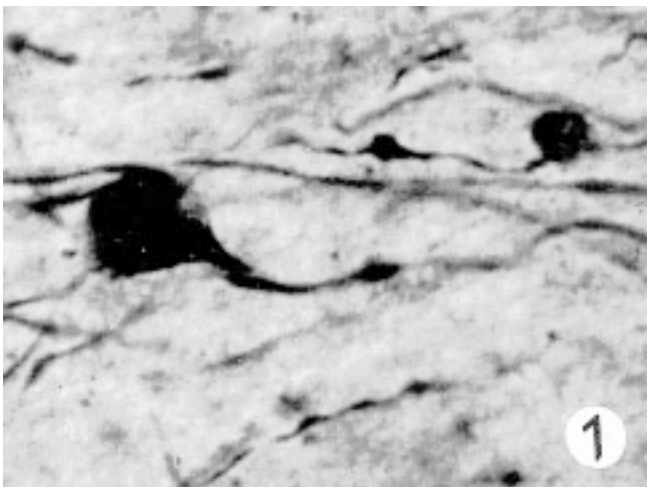

Fig. 1. Cellular (terminal) and axonal (preterminal) degeneration in spinal trigeminal tract nucleus of a dog with paraplegia induced by thirty minutes of spinal cord ischemia and reperfusion. Nauta staining method; magnification 280 ×

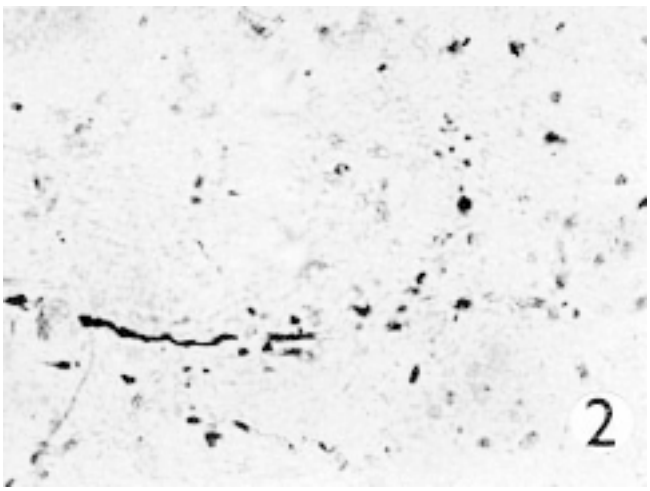

Fig. 2. Several disintegrated and plenty of degenerated cells and axons of spinal trigeminal tract nucleus of a dog with paraplegia induced by ischemia-reperfusion injury of its spinal cord. Nauta staining method; magnification 160 ×

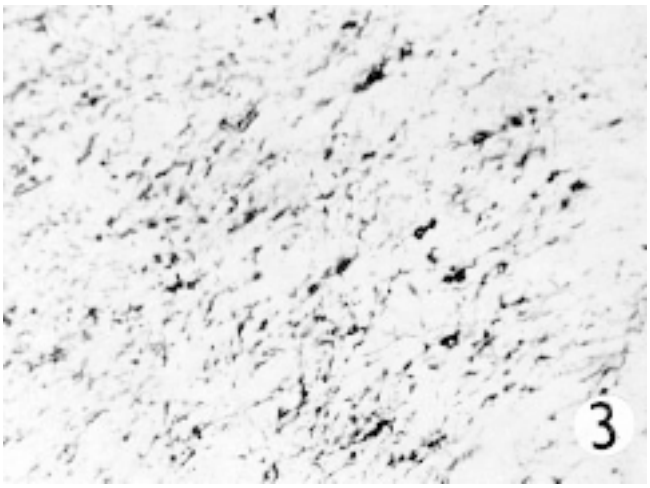

Fig. 3. Densely stained axons localised in dorsolateral spinal fascicle (very probably part of spinocerebellar tract) of a dog after thirty minutes of high-thoracic-aorta cross-clamping and six days survival. Nauta staining method; magnification 160 ×

Administration in Košice (decision No. SK P 53004) and by the Ethical Commission of the Neurobiological Institute of the Slovak Academy of Sciences in Košice. Nine adult mongrel dogs of both sexes, free of heart worm disease, weighing eighteen to twenty-five kilograms were used in the study. The anaesthesia was induced in all animals with pentobarbital (*Pentobarbitalum natricum* – "Pentobarbital", SPOFA, Prague) administered intravenously in a 30 $mg.kg^{-1}$ dose, then intubated with an endotracheal cannula ("Portex", BERCK, Paris) of a diameter 8–12 mm, and placed on a volume-cycled ventilator ("Anemat N 8", CHIRANA, Stará Turá, SR). The anaesthesia was maintained by a mixture of medical oxygen with 1–2 % narcotan (*Halothanum thymolum stabilisatum* – "Narcotan", LÉČIVA, Prague).

The continuous direct monitoring of the arterial blood pressure in a radial artery (monitor LMP 150, TESLA, Piešťany, SR), EKG (monitor LKM 220, CHIRANA, Stará Turá, SR), and arterial blood gases (automatic gas check 995 Hb, CMI, Wien) were performed. The rate of ventilation was adjusted to maintain arterial pO_2 between 80 and 100 mm Hg and pCO_2 at about 38 mm Hg (normal canine levels). The arterial blood pressure (which increased in each dog after the high thoracic aorta cross-clamping) was held at presurgical levels by an infusion of nitroprusside (*Natrii nitroprussidum dihydricum* – "Nipride", ROCHE, Paris).

The dogs were divided into two groups:

1. *Non-ischemic controls* (n = 3). The animals underwent a left-sided thoracotomy through the fifth intercostal space. After sixty minutes of survival they were transcardially perfused with 3000 cc of saline and fixed by the same volume of 10 % neutral formaldehyde.

2. *Ischemia-reperfusion injuries of the spinal cord* (n = 6). The descending aorta, just distal to the origin of the left subclavian artery, was occluded by tourniquet for thirty minutes, then the vessel was released, the thoracotomic wound sutured in anatomical layers and the animal let live for five days (a reperfusion period). On the sixth postsurgical day the dogs were transcardially perfused and fixed in a deep intravenous anaesthesia by pentobarbital in a dose of 50 $mg.kg^{-1}$.

The spinal cords and brains of sham and experimental animals were removed and postfixed with 10 % formaldehyde. Specimens comprising lower thoracic (Th9–Th13) and lumbosacral (L3–S1) spinal cord segments, *medulla oblongata, pons Varoli, mesencephalon* and vermian part of *cerebellum* were cut by a freezing microtome in semithick (30 μm) sections. Then the slices were processed according to the Nauta staining method using the Laidlaw's solution (12).

RESULTS

Changes of spinal cord neurons induced by thirty minutes of ischemia and thirty minutes to six days of

reperfusion have been described in detail elsewhere (15, 16). In this study we have concentrated on *nucleus tractus spinalis nervi trigemini.* Damage to its structures was characterized by the appearance of dark profiles of degenerated axons possessing high Nauta-positivity in the whole caudo-rostral range of he tract.

The analyses of the interrupted series of transversal sections of *medulla oblongata* from the level of the first cervical segment to the anterior border of the spinal trigeminal tract of experimental animals after thirty minutes of thoracic aorta occlusion and six days of reperfusion showed preterminal (axonal) and terminal (cellular) degenerations in the whole caudal part of the *nucleus* (Fig. 1). From the superficially lying fascicle, i.e. from the spinal trigeminal tract, solitary or into small groups situated axons separated. They showed a typical droplet degeneration in longitudinal sections as well. It proved that anterograde degeneration of these axons was complete and was similar to the picture of degenerations in other ascending spinal tracts. Solitary or grouped axons penetrated the layer of *cellulae marginales* and *substantia gelatinosa* without setting collaterals to neurons of these two layers of the nucleus.

Long degenerated axons as afferent parts of caudal portion of spinal trigeminal nucleus were seen as several hundred micrometers long sections. In the next layer, i.e. in *nucleus proprius* they formed a distinct preterminal and terminal plexus with plenty of disintegrated axons (Fig. 2). Typical terminal parts of axons were found in the neuropil, but they only rarely touched the surface of the cells in *nucleus proprius.* A terminal plexus was situated in the whole spinal nucleus and in most slices it imitates (from a microtopographical point of view) the half-moon shape of *nucleus tractus spinalis nervi trigemini.*

Sporadic degenerations, deeper in the *medulla oblongata*, i.e. medially to the spinal trigeminal tract, were also observed. They were scattered among the cells of the lateral strip of the reticular nuclei. Preterminal and terminal degenerations inside the *nucleus tractus spinalis nervi trigemini* occurred bilaterally with the same density and arrangement. Analyses of transversal slices of spinal cord showed that the source of axons leading from the lower thoracic and lumbosacral spinal cord segments into the nucleus of the spinal trigeminal tract were most probably axons from the posterior spinocerebellar tract (Fig. 3).

DISCUSSION

An irreversible paraplegia, which can develop following thoraco-abdominal aortic or principal vessel surgery in spite of the utilisation of different preventive and therapeutic measures, is a devastating complication (1, 19). Numerous clinical and experimental reports have addressed various aspects of this clinical entity, but no definitive solution of the complex problem has yet been found (1, 2, 7, 13, 19). This inspired us to study histopathologic changes of the central nervous system structures induced by thirty minutes of high thoracic aorta cross-clamping (which due to the spinal cord blood supply will cause an ischemic injury) and different time periods of reperfusion-survival thirty minutes to six days (8, 16, 17, 19).

Various experimental models of spinal cord ischemia imitating as much as possible the situation during an aortic or important intrathoracic vessels surgery in humans have been developed. According to preference, equipment and intentions of the authors, different animals ranging from rats and rabbits, to cats, dogs, sometimes even pigs have been used (7, 13, 18—20). Having had a positive experience with the canine experimental model, developed in the Neurobiological Institute of the Slovak Academy of Sciences in Košice, authors have repeatedly used them (16, 17). With the aim of making results comparable, they decided not to change the proven type of anaesthesia for the more up to date type, recommended in current veterinary surgery (10).

The occurrence of preterminal and terminal neuronal changes displayed by means of the Nauta staining method in *nucleus tractus spinalis nervi trigemini* after temporary occlusion of the thoracic aorta followed by reperfusion, represents a surprising localisation of spinal afferents in dogs.

Analyses of terminal projections into trigeminal complex nuclei, performed following precisely accomplished lesions of *ganglion semilunare Gasseri* did not indicate a possibility, that axons other than those originating in the pseudounipolar cells of this ganglion could lead to neurons of a spinal trigeminal tract nucleus (9, 14, 15, 18). Descending branches of the semilunar ganglion receive a precise microtopography, which is the principal condition for the selective cutting of a part of this tract in the neurosurgical treatment of different types of primary facial pain syndromes in humans – i. e. trigeminotractotomy after Sjoeqvist (5, 9, 14).

In connection with distinct terminal changes of neural cells in caudal parts of *nucleus tractus spinalis nervi trigemini* following spinal cord ischemia-reperfusion injury, which we consider to be undoubtedly of spinal origin, an essential question arises about which neurons are the source of axons projecting to the spinal trigeminal tract. It looks as if they are very probably a part of the *tractus spinocerebellaris posterior* (11, 20). In spite of the fact it is not possible to reject some influence of species variability between man and dog, such an existence of spinal projections to the caudal part of *nucleus tractus spinalis nervi trigemini* represents a significant finding, important not only for experimental neuroanatomy, but for human neurosurgery, as well (3—6, 20).

Damage to these spinocerebellar fibres can explain ataxia, balance disturbance and problems with movement coordination observed in patients following Sjoeqvist's operation (5, 14). That is why a trigemino-

tractotomy should not be indicated for professional musicians (especially pianists), dancers, roofers, sportsmen etc. If afflicted by trigeminal, glossopharyngeal or geniculate neuralgia, a different type of surgical intervention must be considered for these individuals (3—6, 15, 18).

CONCLUSIONS

The results of this canine experimental study imply the existence of important connections between some spinal ascending projections (particularly posterior spinocerebellar tract) and structures of the trigeminal complex in dogs. This finding is important not only for experimental neuroanatomy, but should also be taken into consideration during the planning phase of neurosurgical interventions in humans.

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THE EFFECT OF N-ACETYLCYSTEINE ON THE ISCHAEMIC TRIGEMINAL GANGLION

Kalanin, P., Flešárová, S.*

Institute of Biological and Ecological Sciences, P. J. Šafárik University
Šrobárova 2, 040 01 Košice
***University of Veterinary Medicine, Komenského 73, 041 81 Košice**
The Slovak Republic

kalaninpe@stonline.sk

ABSTRACT

We evaluated the influence of treatment with a free radical scavenger N-AcetylCysteine (NAC) on trigeminal ganglion neurons, in a rat model of thirty minutes global brain ischaemia and three days survival. Two experimental groups of animals – ischaemic non treated, and ischaemic preischaemically NAC treated (350 mg.kg^{-1} i.p.) were evaluated by light and electron microscopy. The results obtained demonstrate, that preischaemic NAC administration substantially reduced the occurrence of degenerating small B-cells neurons.

Key words: ischaemia; NAC treatment; trigeminal ganglion

INTRODUCTION

Different types of sensory neurons have been characterized by their sensitivities to chemical, mechanical and thermal stimuli (11). Neurons of cranial sensory ganglia in adult mammals constitute a heterogenous population in terms of the transduction properties of their sensory endings and the quality of the sensation evoked by their activation. They also differ in a number of morphological and functional characteristics, such as size, degree of myelination (3) of the peripheral axon, immunocytochemical properties of the soma, neuropeptide content, and passive and active membrane properties of the cell body (4). Sensitive neurons have been identified in trigeminal ganglia (TG) too (6). TG neurons are involved in the innervation of intracranial blood vessels forming trigeminovascular system which plays an important role in the physiology of brain circulation.

It has been suggested that ischaemia plays an important role in the pathogenesis of many acute and/or chronic diseases of the trigeminal neuralgia (1, 10). There is a growing body of evidence, too, that ischaemic injury of neurons is linked to the production of oxygen-free radicals during the ischaemic and early reperfusion phase. It has been shown, that the use of free radical scavengers enhances neuronal survival (5).

N-AcetylCysteine (NAC) is the amino acid L-Cysteine plus an acetyl (-CO-CH_3) group attached to the amino (NH_2) group. The hydrogen atom in the thiol (ie, -SH) group of many sulfur-containing anti-oxidants can act as an electron for neutralizing free-radicals. NAC as a sulphydryl donor, it contributes to the regeneration of glutatione and it acts through a direct reaction with hydroxyl radicals. NAC research also indicates that this substance can also boost immune system function, specifically, it may help protect the lung tissues, kidney damage, health of the liver, which normally has the job of eliminating toxins. NAC may be permising not only for diabetic neuropathy but other neurodegenerative conditions (9).

In the present study we have evaluated the influence of preischaemic NAC-treatment on ischaemic alterations in trigeminal ganglion neurons after global brain ischaemia and reperfusion in rats.

MATERIAL AND METHODS

All procedures were reviewed by the local animal care committee and were consistent with the IASP guidelines.

Adult male rats (n = 15), weighing between 200 and 220 grams were used in this study: a group of control rats (n = 5); a group of ischaemic nontreated rats (n = 5) and a group of rats injected with a single dose of NAC (350 mg.kg^{-1} i.p.) 30 minutes before the ischaemic insult (n = 5).

Transient global cerebral ischaemia was produced by a modified four-vessel occlusion. Briefly, the animals were anaesthetized intraperitoneally with pentobarbital (40 mg.kg^{-1}) and both their vertebral arteries were cauterized trough the alar foramina at the level of the first cervical vertebra. The common carotid arteries were isolated and polyethylene cuffs were placed loosely around each artery without interrupting blood flow. The animals were allowed to recover for 24 hours. On the next day, the rats were reanaesthetized with 2 % halothane in 70 % nitrous oxide and 30 % oxygen. Ischaemia was produced by the occlusion of both carotid arteries with the atraumatic clips for thirty minutes. After the induction of ischaemia, halothane was removed. Body temperature was maintained at 37 °C with a heating lamp. The rats were allowed to recover and returned to their cages with free access to food and water.

The animals were left to survive for three days, then they were killed by transcardial perfusion with the fixative containing 2 % paraformaldehyde and 1 % glutaraldehyde in 0.12 mol phosphate buffer (pH 7.4). After overnight immersion in the same fixative, the trigeminal ganglia were removed, postfixed in 1 % osmium tetroxide for 2 hours, dehydrated in a graded ethanol series and embedded in Durcupan ACM (Fluka). Semithin sections stained with toluidine blue were used for light microscopy. Ultrathin sections stained with uranyl acetate and lead citrate were examined in an electron microscope Tesla BS 500.

The percentage of alterations in experimental and control animals were compared using one-way ANOVA ($P < 0.05$) and were peformed separately for large light and small dark neurons.

RESULTS

A total of 500 ganglion cells were evaluated in each animal. Within the ganglion neuronal population, a higher vulnerability of B-type neurons was apparent. A-type neurons largely seemed not to be markedly affected. The majority of the neuronal population in the rat trigeminal ganglion consists (Table 1) of small intensively stained B-cells, that contain an evenly distributed Nissl substance. The large light neuronal cells with round centrally placed nuclei and well recognizable Nissl bodies are classified as A-type neurons.

The percentage of altered neurons in non-treated animals at three days post ischaemia was significantly higher in both types of neurons in comparison with the control group. The margination of the Nissl substance and nuclear eccentricity in A-type neurons in control rats reached 4.2 % and 3.6 %, respectively. Both parameter rose to 28 % or 1 % in ischaemic animals. B-type neurons showed similar changes, with an increased proportion of vacuolized perikardia reaching 15 % after ischaemia compared with 2.5 % in the control group.

A similar protective effect of NAC was demonstrated in A-type and B-type neurons with preischaemic NAC administration. Both types cells showed good structural preservation. The NAC pre-treatment led to a significant decrease in the occurrence of vacuolated small (7.5 %) and large cells (3.2 %). In both types of neurons a decrease in nuclear excentricity and Nissl substance margination was noticed. In B-cells significant differences between the NAC-treatment groups of vacuolation and peripheral Nissl displacement were found.

Table 1. Percentage of neuronal changes after ischaemia

	Peripheral Nissl %	Eccentric nucleus %	Vacuolized neurons %
	Large A-cells		
Control (n = 5)	4.2	3.6	0.8
Ischaemia 3 days (n = 5)	28#	12#	6.1#
NAC pretreatment 3 days (n = 5)	17.9#*	7.1#*	3.2#*
	Small B-cells		
Control (n = 5)	5.0	9.9	2.5
Ischaemia 3 days (n = 5)	20.6#	17.4#	15#
NAC pretreatment 3 days (n = 5)	16.3#	14.0#	7.5#*

— $p < 0.05$ compared to control;
* — $p < 0.05$ compared to nontreated animals; one-way ANOVA

DISCUSSION

The aim of the work was to create a model situation during which ischaemia showed the influence of preischaemic NAC-treatment in order to recommend provisions for clinical practice.

NAC is most effective when administered on an empty stomach. NAC is rapidly absorbed and reaches a maximum plasma level in two to three hours, with a half life of about six hours. NAC readily enters cells and is hydrolyzed to cysteine. Regular supplementation with NAC will increase the urinary excretion of copper, zinc and other minerals. Scientists must still determine how much NAC can be safely absorbed and processed by the body.

Evidence indicates, that high doses of NAC can act as a pro-oxidant rather than an antioxidant. In this study was NAC given i.p. at doses 350 $mg.kg^{-1}$. The drug was well tolerated.

The results of the investigation revealed that within the ganglion neuronal population, a higher vulnerability of B-type neurons was apparent. Small B-cells showed degenerative changes with extensive mitochondrial vacuolation. Mitochondrial swelling is linked to a loss of intracellular calcium homeostasis which triggers the production of oxygen radicals. The radicals act as possible mediators of cellular damage (12), leading to structural changes in the cell membranes and other cellular components demonstrated in this study.

Preischaemic NAC application markedly reduced the presence of degenerating B-cells in comparison with non-treated animals. The mechanism of NAC action probably lies in an enhancement of the glutathione and hydroxyl radical scavenging system (9, 10). There are also other possibilities that may account for the neuroprotective influence of NAC. For example a deleterious ischaemia/reperfusion effect on the microvascular and endothelial function (2), leading to the inhibition of endothelial/neutrophil interaction may be ameliorated by NAC application.

Our experiments indicate that rats injected with a single dose of NAC (350 $mg.kg^{-1}$ i.p.) thirty minutes before the ischaemic insult can improve neuron survival in the trigeminal ganglion following thirty minutes global brain ischaemia and three days survival. NAC can reduce the reperfusion injury of neurons.

NAC administration appears to be an efficient intervention which can improve the structural preservation of vulnerable B-cells after ischaemia. This may play an important role in the regulation of cerebral circulation due to the production of vasodilatatory neuropeptides (7, 8), such as substance P and calcitonin gene-related peptide (12). NAC have a value in the treatment of some cerebrovascular diseases. More research in this area is need, however.

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THE PATHOLOGICAL EVALUATION OF SADDLED SEA BREAM *(Oblada melanura)* CAUGHT BY EXPLOSIVES, AND THEIR CHEMICAL AND QUALITATIVE CHANGES DURING STORAGE ON ICE

Ragias[1], V., Govaris[2*], A.
Athanassopoulou[3], F., Sabatakou[4], O.

[1]Ministry of Agriculture, Fisheries and Food, Centre of Veterinary Establishments of Thessaloniki, Institute of Infectious and Parasitic Diseases, Department of Aquatic Organisms Pathology 26th October Str. 80, 54627 Thessaloniki
[2]Laboratory of Hygiene of Foods of Animal Origin, University of Thessaly Faculty of Veterinary Medicine, School of Health Sciences, 221 Trikalon Str., 43100 Karditsa
[3]Laboratory of Ichthyology & Fish Pathology, University of Thessaly Faculty of Veterinary Medicine, School of Health Sciences, 221 Trikalon str., 43100 Karditsa
[4]Institute of Veterinary Research of Athens, NAGREF, 25 Neapoleos Str., Agia Paraskevi 15310 Greece

RUNNING TITLE: Explosive fishing of saddled sea bream

ABSTRACT

Saddled sea bream (*Oblada melanura*) caught by explosive compounds (EXPL group) illegally, were examined for lesions caused to fish by the explosion. The colour of all fish of the EXPL group was a little paler than the normal grey-black colour of saddled sea bream caught by gill net (CONTR group), under normal conditions. In a necropsy of all the examined fish of the EXPL group, internal organs showed hyperaemia, while spleen and liver were haemorrhagic. Since several chromatophore cells were ruptured in all the examined fish of the EXPL group, the examination of chromatophore cells of the saddled sea bream epidermis proved to be an efficient method of detecting fishing by explosives. Both fish groups were stored on ice for three days, and examined for quality and chemical changes. According to the European Union (EU) grading quality scheme, the fish of the CONTR group were evaluated with higher grades than these of the EXPL group either on the first or the third day of storage on ice. The chemical parameters of trimethylamine (TMA), total volatile base nitrogen (TVB-N) and the thiobarbituric acid number (TBA) of the CONTR group were typical for saddled sea bream (*Oblada melanura*) and significantly higher ($P > 0.05$) than those of the CONTR group either on the first or the third day of storage on ice. The rapid quality deterioration of the saddled sea bream (*Oblada melanura*) caught by explosives is another adverse factor of illegal fishing with explosives.

Key words: explosive fishing; quality changes; saddled sea bream *(Oblada melanura)*

INTRODUCTION

The impact of fishing with explosive compounds is catastrophic for fish, living organisms and the marine ecosystem (8, 14). All types of fish (young and old, of high or low commercial value, consumable or inconsumable etc.), falling victim to the explosion. Various organisms are killed, the trophic chain is affected and the life of fish in the sea is endangered. The reef is destroyed, and cannot provide feed and be a shelter for marine organisms; thus, any explosion in

*Corresponding author; E mail: varagias@otenet.gr.

the marine environment can have a direct or a long-term catastrophic effect on marine life. Thus, fishing by explosive compounds has been banned and is considered illegal in most countries of the world. In certain countries (e.g. Canada), when the use of explosives is required for construction purposes, official permission is necessary from the state authorities for the protection of marine organisms (22).

Although fishing with explosive compounds is illegal, it is still practiced irresponsibly in many countries, because it is an easy way to catch fish (14). Nowadays, explosive compounds used for fishing may be dynamite as well as fertilizer chemical compounds such as ammonium and potassium nitrate (NH_4NO_3; KNO_3).

In Greece, when the port police authorities arrest fishermen for illegal fishing with explosive, they deliver the fish to the state laboratories of hygienic control of fisheries for laboratory examination and confirmation of their findings on the fish. Fish caught with explosive compounds may show lesions such as a rupture on the swim bladder, haemorrhagic internal organs such as kidneys, spleen or liver, fractures of the spinal cord and thorax bones, haemorrhagic skin on the belly or damaged fins.

Severe lesions like rupture of the belly skin or damaged fins may be easily seen, if the caught fish are close to the centre of explosion. However, fishermen reject those fish with obvious fish body damage and retain for sale the fish without any lesions, which could be easily recognized by the ordinary consumer. Therefore, the inspection authorities must be very careful during inspection of the fish, which may be caught by explosives. The pathological evaluation of fish may be also very important for the detection of the illegal method of fishing by explosives.

The quality and chemical changes of fish caught by explosive compounds during storage on ice has not been investigated. Thus, the aim of this work was, first the pathological evaluation of saddled sea bream (*Oblada melanura*) caught by explosives, and secondly the investigation of the quality and chemical changes of these fish during storage on ice.

MATERIALS AND METHODS

Fish

Saddled sea bream (*Oblada melanura*) caught by explosives (EXPL) close to Halkidiki peninsula (North Greece), were delivered by the Greek Port Police to the State Veterinary Laboratory of Hygiene of Fish Products at Thessaloniki, Greece. The fish had an average weight of 220 grams and an average length of 170 centimetres. The same number (35) of saddled sea bream (*Oblada melanura*) caught by gill nets under proper conditions and delivered at the fish port of Thessaloniki on the same day, were bought and served as control (CONTR).

All the fish (CONTR or EXPL) were transported to the laboratory in ice and stored without evisceration on ice. Fish from both groups were analysed on the first and third day of storage. The fish were analyzed no longer than the third day due to the low number of fish in the EXPL group (n = 35) delivered by the Greek Port Police to our Laboratory.

Pathological evaluation and microbiological examination

Visual examination was carried out on the external body surface, the gills and the internal organs by methods described by Roberts (16). All sampled fish underwent necropsy. The examination of chromatophore lesions, typical for these cases was carried out as follows: Scales from the abdominal wall (3 × 3 centimetres in size) were carefully removed; then the skin was cut and placed on a slide and covered with a drop of glycerine (5).

For microbiological analysis, kidney and spleen samples were inoculated onto Tryptone Soy Agar (TSA, Oxoid, Basingstoke, UK) and Thiosulphate Citrate Bile Salt Agar (TCBS, Oxoid) for the presence of bacteria, according the methods described by Roberts and Shepherd (17).

Chemical analysis

The determination of trimethylamine (TMA) was carried out according to methods of Simeonidou *et al.* (18). The total volatile base nitrogen (TVB-N) was determined according to a method described by Pearson (13) as modified by Vareltzis *et al.* (21). The thiobarbituric acid number (TBA), expressed as mg malonaldehyde per kg of flesh, was estimated according to a method described by Botsoglou *et al.* (2).

Quality assessment of freshness

Seven fish from each examined fish group were evaluated at each time of sampling by five expert panelists from the laboratory staff, trained in grading fresh fish according to the European Union (EU) grading scheme as described by Howgate *et al.* (7) and Simeonidou *et al.* (19). The appearance of the skin, eyes, gills and internal organs, surface slime, the odours and texture of each fish were assessed into four quality grades of freshness. In this EU grading scheme, excellent quality (perfect condition), high quality (slight loss of excellent characteristics), good quality (some deterioration but fit for sale) and unfit for sale were assigned as E, A, B and C grades, respectively. The total grades of each fish were estimated from the grades attributed by each panelist and the final grade of each fish group was estimated from the fish examined on each day of evaluation.

Statistical analysis

Statistical analysis used the F-test for the comparison of variance and *t*-test for the evaluation of difference in mean values. If the evaluation showed that variances were equal or different the appropriate *t*-test was used at a level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

In the visual examination of the EXPL group, the colour of all fish was a little paler than the normal grey-black colour of fish of the CONTR group, but the differences were not very distinct. In order to find this difference in colour between saddled sea bream caught by gill nets or explosives, the inspector veterinarian must

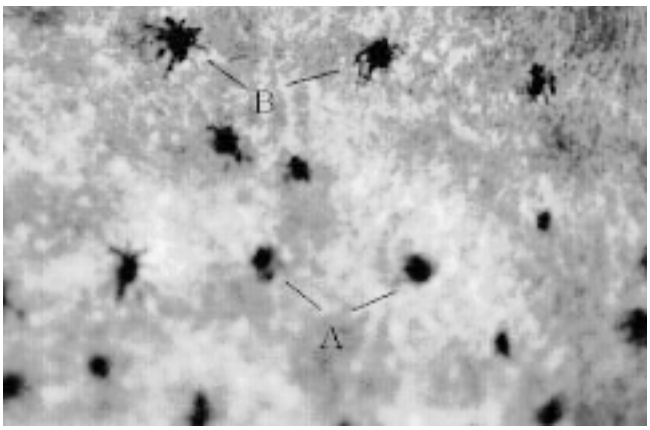


Fig. 1. Chromatophore cells of skin of saddled sea bream (*Oblada melanura*) caught with gill net (CONTR group); A: chromatophore cells in systolic phase B: chromatophore cells in diastolic phase

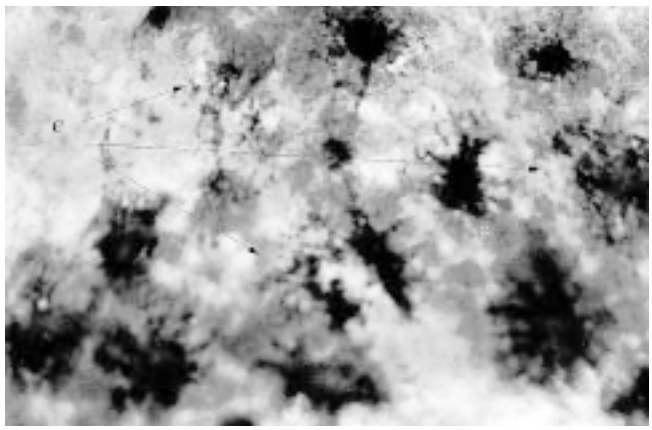
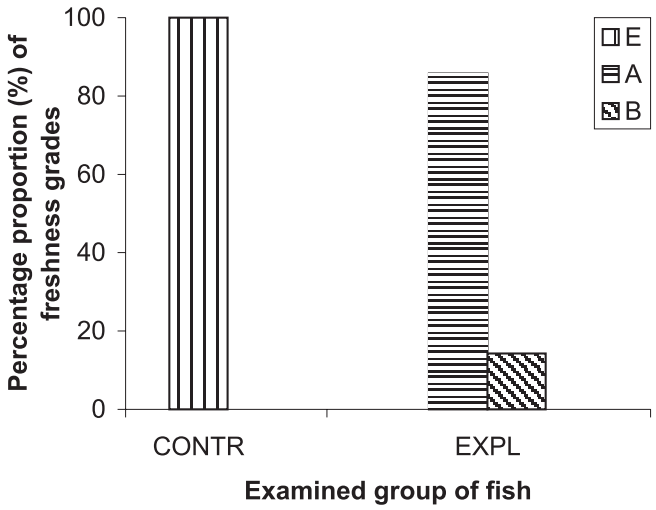
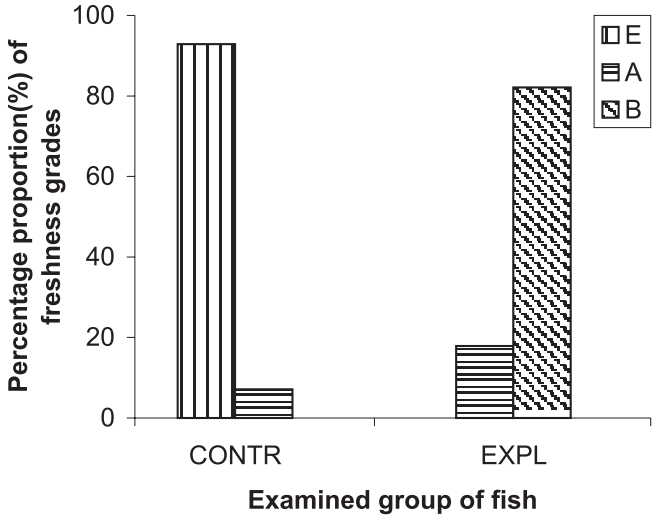


Fig. 2. Chromatophore cells of skin of saddled sea bream (*Oblada melanura*) caught with explosives (EXPL group). Chromatophore cells are ruptured and the melanin is dispersed peripherically (C, *arrow*)



E — excellent quality; A — high quality; B — good quality

Fig. 3. Freshness grading of saddled sea bream (*Oblada melanura*) caught by means of net (CONTR) or explosives (EXPL) according to EU grading scheme during the first day of storage on ice (n = 28)



E — excellent quality; A — high quality; B — good quality

Fig. 4. Freshness grading of saddled sea bream (*Oblada melanura*) caught by means of net (CONTR) or explosives (EXPL) according to EU grading scheme during the third day of storage on ice (n = 28)

be very careful and experienced in fish evaluation. For 5.7 % of the examined fish, a small area of haemorrhagic abdominal skin was observed, located in front of the anus.

In the necropsy of fish of the EXPL group, the internal organs showed hyperaemia, while spleen and liver were haemorrhagic in all the examined fish. A rupture of the swim bladder was observed in 8.57 % of the examined fish. In contrast, such lesions were not observed in the necropsy of the CONTR group.

Several chromatophore cells of the epidermis were destroyed and melanin was arranged in a circular manner, leaving a round centre in all the examined fish of the EXPL group (Fig. 1). In contrast, fish from the CONTR group did not show any of the above lesions (Fig. 2**).** The chromatophore cells are migratory, amoebic-like cells that have in their cytoplasm granules of melanin. They are responsible for the body colour and are found in the fins, on the head, the dorsum and body sides. The colour changes by means of movement of melanin inside the cells either with dispersion radically in the cytoplasm (resting/diastolic phase) or by concentration of melanin in the centre (acting/systolic phase).

This procedure is monitored by the nervous system through secretion of hormones. When explosion occurs, the chromatophores cells are ruptured and the colour is dispersed in the cell nucleus. According to our results the examination of chromatophore cells of the saddled sea bream epidermis proved to be an efficient method of detecting fishing with explosives and this is in accordance with previous results for fishing with explosives (15).

The microbiological analysis revealed that no bacteria were present in the liver and spleen of fish in both groups, indicating that the lesions of the EXPL group were not associated with any growth of pathogenic bacteria.

Figure 3 shows the freshness grading, according to the EU grading scheme of saddled sea bream (*Oblada melanura*) of the CONTR or EXPL groups on the first day of storage on ice. As shown in this figure, all of the saddled sea bream (*Oblada melanura*) of the CONTR group were evaluated as excellent grade E (100 %), which is ranked first in this grading scheme.

In contrast, none of the saddled sea bream (*Oblada melanura*) of the EXPL group were evaluated as excellent grade (E), and this may be due to the haemorrhage of the abdominal skin in front of the anus and changes in the internal organs. Thus, fish from the EXPL group were evaluated by the panellists as high grade A (85.7 %) and good quality B (14.3 %). It is important to note that grades A and B of this EU grading scheme are ranked second and third, respectively.

Figure 4 shows the freshness grading, according to the EU grading scheme, of saddled sea bream (*Oblada melanura*) of the CONTR or EXPL groups on the third day of storage on ice. The results indicated that fish from the CONTR group did not show important changes in their freshness grades on the third day, as compared to the first day of storage on ice. Thus, 92.8 % of the fish were evaluated as excellent grade E and only a 7.2 % as high quality grade A. In contrast, the saddled sea bream (*Oblada melanura*) caught with explosives (EXPL) showed high changes in their freshness grades. Thus, 17.85 % of the fish of this group (EXPL) was evaluated by the panelists as high quality grade A and a 82.14 % as good quality B.

Table 1 shows the changes in trimethylamine (TMA), the total volatile base nitrogen (TVB-N) and the thiobarbituric acid number (TBA) of saddled sea bream (*Oblada melanura*) of the CONTR or EXPL groups during the three days of storage on ice. The TMA values for the fish of the CONTR group were low and were not significantly changed ($P > 0.05$) from the first day to the third day of storage.

As known, TMA is derived from the decomposition of trimethylamine oxide (TMAO) of the sea fish and is mainly caused by the enzymic action of bacteria grown on the fish, although a small proportion may be derived from the action of endogenous enzymes of the fish (6). At the beginning of storage on ice a low amount of TMA was determined in fish of the Sparidae family (10; 3) as well as in other sea fish (9, 11, 12, 19).

The TMA values of fish of the EXPL group were significantly changed ($P < 0.5$) from the first day to the third day of storage on ice. The TMA values of the fish of the EXPL group were also significantly ($P > 0.05$) higher than these values of the CONTR group either on the first or the third day of storage. This fact implies a higher skin microbial growth on the sea bream (*Oblada melanura*) of the EXPL group than on the CONTR group and their enzyme action accelerated the rate of decomposition of trimethylamine oxide (TMAO) to TMA.

The microbial growth may be assisted by the damage of the skin, which was visible as skin haemorrhage on certain fish, as reported earlier. The increase in trimethylamine of the sea bream (*Oblada melanura*) of the EXPL group may explain the stronger "fish smell" of these fish as compared to this of the fish of the CONTR group.

The TVB-N values of the fish of the CONTR group were significantly increased ($P < 0.05$) from the first day to the third day of storage. The total volatile basic nitrogen (TVB-N) of the fish flesh is derived from the increase in TMA, dimethylamine (DMA), ammonia and nitrogen compounds.

In contrast to TMA, the total volatile basic nitrogen (TVB-N) shows an increase in higher or exponential rates in most of the fish stored under refrigeration (1.6). Similar changes in TVB-N have been observed by Koutsoumanis and Nychas (10) during storage o gilthead sea bream (*Sparus aurata*) of the same family (Sparidae) on ice.

The TVB-N values of fish of the EXPL group were significantly changed ($P < 0.05$) from the first day to the third day of storage on ice. The TVB-N values of the fish of the EXPL group were also significantly ($P < 0.05$) higher than those values of the CONTR group either on the first or the third day of storage.

Table 1. Changes in trimethylamine (TMA), total volatile base nitrogen (TVB-N) and thiobarbituric acid number (TBA) of saddled sea bream (*Oblada melanura*) caught by means of net (CONTR) or explosives (EXPL) during storage on ice

Days of storage	TMA* (mg N.100 g^{-1})		TBA* (mg MA.kg^{-1})		TVB-N* (mg N.100 g^{-1})	
	(CONTR)	(EXPL)	(CONTR)	(EXPL)	(CONTR)	(EXPL)
1	0.17 + 0.02a	0.28 + 0.07b	0.26 + 0.03a	0.63 + 0.08b	18.22 + 0.46a	22.4 + 0.38a
3	0.18 + 0.01a	0.44 + 0.01b	0.29 + 0.06a	0.94 + 0.02b	20.10 + 0.19a	26.8 + 0.52b

* — Mean values + standard deviation. Mean values within a row under the same chemical parameter followed by the same letter are not significantly different ($P < 0.05$)

The results showed that an increase in, not only trimethylamine but, the total volatile basic nitrogen of the flesh of these fish as compared to that of the CONTR group. The increase in TVB-N may be primarily due to the skin microbial growth on the fish and in a lesser manner to the action of the enzymes endogenous to their tissues (4, 9, 12).

The TBA values of the fish of the CONTR group were not significantly changed ($P > 0.05$) from the first day to the third day of storage and this indicates the low oxidative activity in the fish flesh during the first days of storage on ice. The TBA values determined for the fish of the CONTR group are in agreement with the values determined by other researchers for fish that belong to Sparidae family (3, 20).

The TBA values of fish of the EXPL group were significantly changed ($P < 0.05$) from the first day to the third day of storage on ice. As for TMA and TVB-N values, the TBA values of the fish of the EXPL group were also significantly ($P < 0.05$) higher than those values for the CONTR group either on the first or the third day of storage.

This fact indicates a higher oxidative action on the fish of the EXPL group than on the fish of the CONTR group. This may be explained from the cell rupture because of the action of the blast wave of the explosives, which resulted in the release of endogenous enzymes with oxidative action from the cells.

An increase in the oxidative action of fish flesh was also observed in other cases with cell rupture of the muscle tissue like filleting or cutting of flesh or even evisceration of the fish either during refrigerated (3, 12, 19) or frozen (18) storage of the fish.

In conclusion, saddled sea bream (*Oblada melanura*) caught by means of explosives showed internal organ hyperaemia, while the spleen and liver were haemorrhagic. Since several chromatophore cells were ruptured in all the examined fish of the EXPL group, the examination of chromatophore cells of the saddled sea bream epidermis proved to be an efficient method of detecting fishing by explosives. Fish in the EXPL group were assessed with lower quality grades, according to the EU grading scheme during the three days of storage on ice, than the saddled sea bream (*Oblada melanura*) caught regularly by means of net.

The chemical parameters of trimethylamine (TMA), the total volatile base nitrogen (TVB-N) and the thiobarbituric acid number (TBA) indicated that the saddled sea bream (*Oblada melanura*) caught by explosives had a higher rate for the onset of spoilage during the three days storage on ice than the saddled sea bream (*Oblada melanura*) caught regularly by gill nets. The rapid quality deterioration of the saddled sea bream (*Oblada melanura*) caught by explosives is another adverse factor in the illegal fishing with explosives.

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THE EVALUATION OF THE PREMI®TEST USED FOR THE DETECTION OF SULPHADIMIDINE RESIDUES IN EGGS

Marcinčák, S.[1*], Hussein, K.[1], Popelka, P.[1], Zdolec, N.[2]

[1]Department of Food Hygiene and Technology
University of Veterinary Medicine Komenského 73, 040 01, Košice
The Slovak Republic
[2]Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55
Croatia

marcincak@lycos.com

ABSTRACT

In our experiment, the presence of sulphadimidine residues in the eggs of laying hens after *per os* administration of Sulfadimidin PG plv. sol. ad us. vet. (PharmaGal, Nitra, The Slovak Republic, 120 mg.hen.day^{-1}) was investigated. Detection of sulphadimidine residues was performed by the fast, reliable screening Premi®Test and the results were confirmed by HPLC. The Premi®Test detected positive results for the presence of sulphadimidine residues eight days after the completion of administration. The last positive results confirmed by HPLC were also recorded on the eighth day (0.096 mg.kg^{-1}). Finally, a correlation between results of Premi®Test and HPLC was recorded.

Key words: egg; HPLC; Premi®Test; sulphadimidine residues

INTRODUCTION

In veterinary medicine, substances with an antimicrobial effect are wide-spread used (9). Sulphonamides are one of the oldest group of pharmacologically active substances used in veterinary medicine to date. Their discovery, in 1935, signified the beginning of a new period in the treatment of various microbial diseases and protozoan infections. At the present time, the development of new wide spectral antibiotics and the higher rate of resistance to sulphonamides has meant that sulphonamides are used rarely for preventative purposes. However, in the treatment of coccidiosis and infections of the digestive tract in poultry potential sulphonamides remain as an effective tool for the elimination of coccidiosis (5).

The occurrence of inhibitory substances and residues of veterinary drugs in food of animal origin is systematically monitored from the veterinary and human medicine point of view (10). Residues of anticoccidials in food of animal origin represent risks of direct and indirect jeopardy to human health and they have a negative impact on technological processes in the food industry. From the point of view of the threat to consumer health, antibiotics used in animals are included in the food chain and cause the creation of resistance and allergy (6,12). Sulphonamides are known also for their negative effects on the thyroid gland in relation to the development of thyroid gland tumours (1).

The effectiveness of the control of food of animal origin must be increased to eliminate health risk to consumers, the negative impact on the environment and technological problems in production (4).

Therefore the availability of simple and reliable screening systems for the detection of antibiotics is an essential tool in assuring the safety of food products. Recently a new broad spectrum screening test for the detection of antibiotic residues and sulphonamides, Premi®Test, has been developed (8, 9). The test was developed originally as a broad-spectrum microbial screening test intended for the detection of antibacterial compounds in meat and meat products. Later it was validated for the screening of additional products of animal origin, such as milk, eggs and honey (14)

In our experiment, the presence of sulphadimidine residues in eggs of laying hens was detected by Premi®Test. The results have been confirmed by HPLC.

MATERIAL AND METHODS

Methanol, acetonitrile, n-hexane, ethyl acetate and acetic acid were purchased from Merck company (Darmstadt, Germany). Sulphamethazine sodium salt (Sigma S 5637), as a standard of sulphadimidine, was taken from Sigma company (USA). Anhydrous sodium sulphate, sodium chloride and sodium acetate were derived from Lachema (Brno, Czech Republic). De-ionised water and all chemicals have p. a. purity of HPLC grade.

A separate stock solution of twenty-five milligrams per twenty-five milliliters (1 $mg.ml^{-1}$) of sulphadimidine was prepared by dissolving sulphamethazine sodium salt in methanol. Working standard solutions (WSS) of sulphadimidine were prepared by the dilution of a stock solution in the following concentrations: 100; 50; 20; 10; 5; 1; 0.5; 0.1; and 0.05 $\mu g.ml^{-1}$. WSS were added to the egg melange (spiking samples). Each concentration was tested three times to determine the limit of detection (LOD) of the Premi®Test.

The Premi®Test was taken from DSM (Netherlands) and Thermoblock (Biotech, The Slovak Republic) was used as a block heater for Premi®Test ampoule incubation. The Premi®Test ampoule method for antibiotic residue detection utilises a culture medium containing *Bacillus stearothermophilus* var. *calidolactis*. The Premi®Test combines the principle of the agar diffusion test with the colour change of the indicator resulting from the active metabolism of the tested microorganism. Homogenised egg fluid (100 µl) was transferred onto the agar in the ampoule, incubated for twenty minutes at room temperature for a predifussion and then released. Ampoules were placed in a water bath at 80 °C for 10 minutes. After this heat pre-treatment the ampoules were incubated for three hours at 64 ± 1 °C and the change of colour was evaluated. Results were obtained by recording the colour at 2/3 of the tube height. A yellow colour indicated the absence of sulphadimidine residues while a purple colour implied the presence of sulphadimidine residues at or above the limit of detection.

A liquid chromatography method (13) with UV detection at 265 nm was used to determination of sulphadimidine residues in egg. Limit of detection (LOD) and limit of quantification (LOQ) is 0.009 $mg.kg^{-1}$ respectively 0.02 $mg.kg^{-1}$.

In our experiment, twenty laying hens (ISA Brown) in the 35th week of laying under veterinary control were used. Laying hens were bred separately in cages and they were fed, *ad libitum.* The feeding mixture HYD-10 (Tajba, Čaňa, The Slovak Republic), free of antibiotics was used. Sulfadimidin PG pl. sol. ad us. vet. (PharmaGal, Nitra, The Slovak Republic) in a dosage of 120 mg (dissolved in water) per kg of body weight and per day with a probe into the oesophagus was administered. Sulphadimidine was applied in the following ways: three days of administration, three days break and three days of drug administration. Six laying hens free of residues were used as a control. The eggs were collected, signed, and stored at 4 °C during fifteen days of withdrawal period from Sulfadimidin PG pl. sol.

Statistical analysis was performed by the statistical program Graph Pad Prism version 3.0 (2000) The results are expressed as an arithmetical mean ($\bar{x}$) and standard deviation (± SD). The HPLC method of sulphadimidine residues detection was statistically analysed by Student pair *t*-test ($P < 0.05$).

RESULTS

The results of LOD of Premi®Test for selected concentrations of the standard mentioned in Material and Methods are recorded in Table 1.

Table 1. The evaluation of the limit of detection (LOD) of the Premi®Test for selected concentrations of sulphadimidine (SM)

SM ($mg.kg^{-1}$)	Results
100	+
50	+
20	+
10	+
5	+
1	+
0.5	+
0.1	+
0.05	+
0.01	–

+ — positive result; – — negative result

Table 2. The determination of the presence of sulphadimidinee residues (SM) in eggs by the Premi®Test during the 15 days withdrawal period from sulphadimidine

Day of withdrawal period	Premi®Test	
	Sample with SM	Control without SM
1	+	–
2	+	–
3	+	–
4	+	–
5	+	–
6	+	–
7	+	–
8	+	–
9–15	–	–

+ — positive result; – — negative result

On the basis of the results presented in Tables 2 and 3, administration of Sulfadimidin PG pl. sol. in dosage 120 $mg.kg^{-1}$ with a probe (according to producer recommendation) caused the rapid occurrence of drug residues in egg content.

Table 3. The concentration of sulphadimidine residues (SM, mg.kg^{-1}) detected by HPLC during the 15 days withdrawal period from sulphadimidine

SM mg.kg^{-1}	Day of withdrawal period 1	2	3	4	5	6	7	8	9—15
$\bar{x}$ ± SD	33.84 ± 3.25	31.86 ± 2.95	1.72 ± 0.33	1.55 ± 0.15	1.11 ± 0.10	0.55 ± 0.05	0.11 ± 0.01	0.096 ± 0.01	0

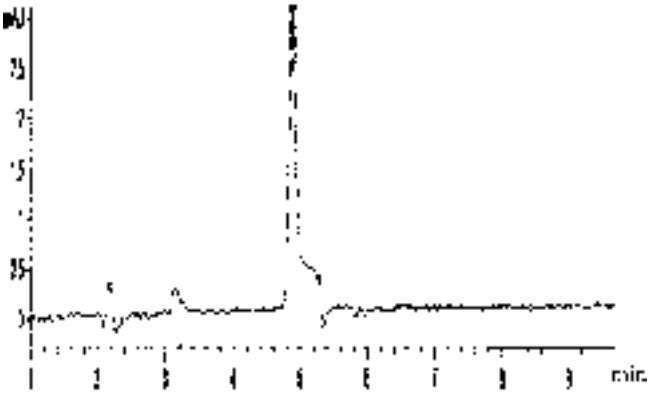

Fig. 1. The chromatographic record of sulphadimidine obtained from eggs examined (first day of withdrawal period)

HPLC conditions: Chromatographic column: Phenomenex RP, C18, 150 — 4.6 mm (5 ěm), flow rate: 1.0 ml.min-1, UV detection: 265 nm, volume: 20 ěl; * — sulphadimidine

The monitoring of sulphadimidine residues by the Premi®Test after administration of Sulfadimidin PG pl. sol. during the withdrawal period are recorded in Table 2. During the first eight days of the withdrawal period the presence of sulphadimidine residues above the detection limit of the Premi®Test (0.05 mg.kg^{-1}) was detected. From the ninth day of the withdrawal period all samples were negative.

The chromatographic record of sulphadimidine from the eggs examined (first day of withdrawal period) by HPLC is drawn in Figure 1. Analytical HPLC separations were performed by a Hewlett Packard machine (model 1050 HP) equipped with autoinjector and a variable-wavelength UV detector. Sulphadimidine was detected by an isocratic system in 4.9 to 5 minutes. The chromatographic column Phenomenex RP C_{18} (150 × 4.6 mm, 5 µm) was used. The mobile phase [acetonitrile/acetate puffer (pH 4.6); 25/75;v/v] was used for the elution of sulphadimidine at 265 nm wavelength. Maximal absorbance of sulphadimidine is in accordance with the established wavelength.

The results of sulphadimidine residues detection by HPLC (during withdrawal period) are presented in Table 3. The first and second days after the completion of sulphadimidine administration high residue concentrations in eggs were recorded (33.84 ± 3.25, and 31.86 ± 2.95 mg.kg^{-1}). On the third day of the withdrawal period a rapid decrease of the residue concentration of sulphadimidine in eggs was found (1.72 ± 0.33 mg.kg^{-1}) with significant statistical difference in results ($p < 0.05$). All these results were above the level of maximum residue limit (MRL, 0.1 mg.kg^{-1}) established by *European legislation* (3).

A significantly statistical decrease of sulphadimidine residue concentration was recorded from the third to the seventh day of the withdrawal period ($p < 0.05$). On the seventh day of the withdrawal period the residue concentration of sulphadimidine was in all samples 0.110 ± 0.10 mg.kg^{-1}. This value is still above MRL. Values of sulphadimidine concentration in eggs less than MRL were obtained on the eighth day of the withdrawal period.

Comparison of the Premi®Test and HPLC showed correlation between both methods and the HPLC method also detected positive results up to the eighth day after the completion of administration.

DISCUSSION

The solution of problems related to the occurrence of inhibitory substances requires better attention to the control of the current higher authority in the evidence from treated animals and the keeping of withdrawal periods valid for individual drugs (7). An important point is the correct use of screening methods intended for the control and identification of inhibitory substances in food of animal origin. The correct use of Premi®Test leads to a decrease in the number of positive animals and their products at the beginning of the food chain and improves the health status of the final consumer (1).

The Premi®Test integrates a strategy of detection of antibacterial substances below MRL for a wide spectrum of biological matrices including eggs. Conventional tests such as the Four-plate test require incubation overnight, and in contrast the Premi®Test provides reliable results within three hours of incubation (14).

Then results of the determination of sulphadimidine residues during the fifteen days withdrawal period are described in Table 2. Over the first eight days of withdrawal period, established for eggs, residues of sulphadimidine were found in concentrations higher than the detection limit of the Premi®Test (0.05 µg.kg^{-1}). The results obtained by the Premi®Test were also confirmed by the

quantitative HPLC method. The results showed that the Premi®Test could be used to detect residues of sulphadimidine in eggs of laying hens. The reliability and sensitivity of the Premi®Test for the detection of sulphonamide residues has also been described by S t e a d *et al.* (14).

The Premi®Test introduces an important tool for the control of the presence of concentrations of inhibitory substance residues over the limit. The detection limit of the Premi®Test for sulphonamides achieved by the authors (2, 14) vary from 0.01 to 0.05 $mg.kg^{-1}$, and the sensitivity of the test is in accordance with the requirements of *European legislation* (3).

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IRON DEFICIENCY IN SUCKLING PIGLETS: PARENTERAL AND ORAL IRON ADMINISTRATION TO PIGLETS (A Review)

Svoboda, M., Drábek, J.

Faculty of Veterinary Medicine
University of Veterinary and Pharmaceutical Sciences Brno
Palackého 1-3, 642 42 Brno
The Czech Republic

svobodama@vfu.cz

ABSTRACT

The review presents data on the mechanism of action, recommended dosages, possible adverse effects and application forms of different preparations both for parenteral and oral administration.

Key words: chelates; iron-dextran; iron salts; iron toxicity

INTRODUCTION

The use of new genetic lines of pigs and management changes have resulted in re-examination of Fe administration to young pigs in terms of efficacy, long-term benefit and freedom from toxicity. Through the years, different forms of iron supplementation have been used in order to prevent the development of iron deficiency anaemia in piglets. Parenteral or oral preparations for iron supply are available.

PARENTERAL ADMINISTRATION

Mechanism of action

The toxicity of the iron preparation must be low, since a high dosage has to be administered when the piglets are about three days old without regard to individual weight. In order to reduce toxicity the iron has to be bound to a stabilizer, which can combine with the iron to give some form of complex. All these iron complexes are of high molecular character. Preparations containing iron-dextran, hydrogenated iron-dextran, dextrin-ferric oxide complex and polysaccharide-iron complex, have been reported as being useful in preventing piglet anaemia (11).

Another substance for intramuscular iron application is gleptoferron. Gleptoferron is a sterile colloidal solution of beta-ferric oxyhydroxide and dextran glucoheptonic acid. The results obtained by Pollmann *et al.* (63) and Vermeer *et al.* (86) have demonstrated that the iron from iron dextran and gleptoferron is utilized with similar efficiency for anaemia prevention in young pigs.

From the injection site a significant part of iron-dextran is taken up by the reticulohistiocytic system. The dextran component is decomposed by lysosomal enzymes of phagocytes and stored as ferritin (41). The elimination of Fe-dextran by reticulohistiocytic system is rapid. In a study conducted by Sladic-Simic and Cvetkovic (71), it has been found that all iron was eliminated from injection site seven days after administration. After an intramuscular injection the Fe content of the inguinal and iliac lymph nodes increased very much and that of the intestinal lymph nodes was raised. The augmentation of the Fe content in the lymph nodes is induced by the ingestion of Fe-dextran by macrophages. The capacity of the immune system is in this way apparently reduced for some time (46). Only cca 1—2 % of Fe from iron-dextran is present as free Fe and binds directly to transferrin (45).

Upon reaching the blood stream the free iron is chelated with an amino acid compound, transferrin. Since even a small quantity of free metallic iron in the blood is toxic, one of the

functions of transferrin is to make certain that the iron in the blood is chelated, thus preventing this toxicity. The other major function of transferrin is to move iron to the areas of need (59).

The binding to the protein carrier is also necessary because free iron ions would be removed by glomerular filtration (47).

With an iron overdose, as for instance with an iron injection or single high iron oral dose, the excess iron is stored in different organs in the form of ferritin (mainly in the liver, spleen and bone marrow) as a readily utilizable resource (6).

The reaction of red blood cell parameters to iron-dextran treatment is rapid. In a study conducted by Holter *et al.* (34) injection with iron-dextran to anaemic piglets led to a rapid and statistically significant increase of Hb, PCV, and MCV four days after the treatment.

Sjaastad *et al.* (70) have found that iron-dextran injection stimulates erythropoietin production. In a study conducted by Sweigert *et al.* (80) the injection of iron dextran at day 3 prevented a decrease in the concentration of retinol in the plasma as was observed in piglets with no iron supplementation. Because of the overall role of vitamin A in growth and tissue and mucosal integrity, this could be of importance for the well-being of the developing piglet.

Dosage

A dose of iron-dextran should ensure that every piglet has sufficient iron to maintain its blood haemoglobin concentration above 100 $g.l^{-1}$. As long as it either begins to take creep feed or is weaned at three to four weeks of age (45).

However the recommendation given by different authors on the dosage and number of injections are not always in agreement.

The most common approach to the prevention of iron deficiency anaemia in baby pigs is to inject 200 mg iron as iron-dextran on the third day of life (11, 85). A total dose of 200 mg is usually recommended as being required to avoid clinically manifest iron-deficiency anaemia, but in order to avoid any chance of a subclinical deficiency the feed should contain additional iron at a minimum level of 240 $mg.kg^{-1}$ (64). In studies conducted by Daykin *et al.* (13) and Dilov and Chakurov (16) the single dose of 100 mg Fe per pig in the form of iron-dextran was not sufficient to produce an optimal antianaemic effect and resulted in lower weight gain compared to 200 mg Fe.

Contrasting information is that one injection of 100 mg of iron is adequate for baby pigs (42). In this study the piglets were weaned at about three weeks of age, at which time those given 200 mg of intramuscular iron between the first and fourth day after birth had higher values in all haematological components. However, there was no significant difference between the mean daily weight gains of the two groups, and 100 mg iron maintained haematological components above levels associated with anaemia. Kay *et al.* (42) concludes that proprietary brands of creep feeds contain a high concentration of iron which may reduce the requirement for iron in the form of iron dextran.

Conflicting results were obtained with multiple iron-dextran injections. Radostis *et al.* (64) concludes that multiple injections give better haemoglobin levels but do not improve weight gain and thus a second injection at two to three weeks of age may not be economical). According to Hill *et al.* (31) one injection of iron (200 mg) given to piglets seems to be adequate to sustain average daily gain from birth through the nursery phase. A second injection of iron does not seem to improve weight gain.

On the other hand Kamphues *et al.* (40) have demonstrated the positive effects of 2nd iron dextran injection (200 mg Fe) at the 21st day of life on performance before and after weaning, especially in piglets with high growth intensity. The second iron injection should not exceed 200 mg otherwise growth is influenced negatively.

In a study conducted by Egeli and Framstad (19), the piglets injected with 180 mg iron dextran on day 1 and having access to extra iron and pelleted feed had relatively low haemoglobin values on days 21 and 35. This indicates that iron reserves at these times after birth were beginning to be depleted.

The differences found between various authors could possibly be explained by different experimental conditions. According to Steinhardt *et al.* (76), requirements for iron supplies can be influenced by the conditions given at any specific pig production unit (nutrition and keeping of sows and piglets, severity and incidence of inadequate foetal iron reserves, actual increase of live weight, consumption of supplementary feed).

Adverse effects of iron-dextran injection

With regard to the wide use of parenteral iron application, undesirable effects occur only seldom. The undesirable effects of iron-dextran injection can be divided into five groups.

a) Morphological-structural alterations

Histological and enzyme-histochemical investigations of the muscle and connective tissue following i.m. iron injection revealed that the degree of possible tissue damage depends on the quality of the administered iron preparation. The morphological-structural alterations from high quality iron preparations are completely restored two to three months after application (75).

b) Iron toxicity

Reports of toxicity after iron-dextran injection in newborn piglets are sporadic, and the risk is not high, however, toxicity does occur.

Sporadically free iron can be released from the preparation too rapidly. It may occur with too high a dose of preparation or as a result of the bacterial decomposition of iron dextran macromolecule, that causes the release of ionized iron. Bacterial contamination can occur with nonsteril application (30). Iron toxicosis can be caused also by a poor quality of Fe-dextran preparation (4).

Acute poisoning usually occurs if the piglets are deficient in vitamin E and selenium. In normal piglets the iron-dextran compounds are safe and are usually not toxic even with repeated injections (64). After injection with iron-dextran under conditions of vitamin E deficiency, even the release of a small amount of iron ions is of importance (45). The antioxidative property of vitamin E is well-known (60). Doug-

herty *et al.* (17) have reported that supplemental dietary vitamin E was essential to prevent mortality in rats given an intraperitoneal injection of Fe.

The most probable background to the reduced resistance against iron is that an animal with a vitamin E and selenium deficiency cannot counteract peroxidative reactions, even when the animal is not extremely peroxidatively challenged (53, 62).

Because free iron can catalyze free radicals from molecular oxygen and hydrogen ions, it can have disastrous consequences for biological materials. Iron can damage tissues by catalyzing the conversion of hydrogen peroxide to free radicals then may attack cellular membranes, proteins and DNA (32, 50). The free radicals induce peroxidation of nonsaturated fatty acids in cell membranes which leads to cell membrane damage (30, 58).

Patterson *et al.* (61) have demonstrated an increase of lipid peroxid levels in the gluteal muscles of piglets which died after Fe-dextran injection.

According to Patterson *et al.* (61), Tollerz (84), Avram *et al.* (4), Wilhelm (89) the affected piglets have increased concentrations of potassium in blood serum, which is caused by the release of K from damaged muscles. The increased blood potassium level interferes with heart action and the hyperkalaemia can even lead to death due to cardiac arrest. After iron dextran injection any excitation of the piglets should be prevented, because a latent disturbance of heart function can be present. The excitation can intensify such a disfunction and even to lead to death (45).

The death of piglets after parenteral iron dextran treatment can be attributed in part to iron toxicosis, in part bacteraemia. Piglets from the same litter can be affected by both the iron toxicosis and bacteraemia. The symptoms of iron toxicosis set in twenty to thirty minutes or four to six hours at the latest after iron treatment, while bacteraemia took two to three days to develop (79).

The affected piglets become listless and inappetent after treatment, and subsequently developed dyspnoea, heart failure, tremor, muscle spasms, movement incoordination, paralysis, followed by a comatose state in the terminal stage. Disease incidence is usually limited to a few litters, the whole litter being affected (30, 79, 89).

The most important precipitating factor in iron toxicosis is vitamin E or selenium deficiency of the sow, which results in deficiency in the piglets (89).

Only a limited and insufficient amount of vitamin E can be transported through a sow's placenta even if the dietary concentration fed to a gestating animal is high, but colostrum and milk concentration can be increased when the nutrient is fed to sows (53).

Therefore immediately after birth the piglets are more sensitive to Fe-dextran injection compared to two day old piglets. This is caused by a low content of vitamin E in blood plasma and tissues in one day old piglets (45, 53).

The plasma biological antioxidant status of piglets is increased by the second day of age, especially in vit. E (nearly a 20-fold increase), because during the first 48 hours of life piglets receive a significant amount of vitamin E from the colostrum (45, 53).

Although the iron-dextran injection on day 1 gives significantly higher haemoglobin concentration on day 7 compared to application on day 3 (23), the i.m. iron application is not recommended earlier than on 3th day of life (45).

Any history of vit. E deficiency in the herd, or previous evidence of iron toxicity is reason to delay iron administration until ≥ 24 hours after administration of vitamin E (89).

The concentration of vitamin E in the colostrum and milk of sows depends on its content in the sow's feed. The occurrence of vitamin E deficiency in sows is rare, because cereal-based feed mixtures have a high content of vitamin E. Vitamin E deficiency occurs more commonly when feed has a high content of readily peroxidized lipid sources, such as polyunsaturated fatty acids (30, 37, 45).

Colostrum is a good source of vitamin E in sows fed an adequate amount of vitamin E. *Colostrum* has a greater concentration of vitamin E and Se than milk (53).

Heinritzi and Plonait (30) recommend that in problematic herds, the piglet should be injected with vitamin E (10—20 mg) a day before iron administration. In a study conducted by Tollerz (84) the vitamin E injected to piglets in doses of 20 to 60 mg per kg 24 hours before iron-dextran injection prevented any death and adverse effects.

There are different recommendations given by various authors on vitamin E administration to sows. Pehrson *et al.* (62) have reported that sows given 1.5 g vitamin E intramusculary seven and two days before farrowing had a higher vitamin E concentration in the colostrum than untreated sows. It resulted also in higher serum vitamin E concentration in the piglets of treated sows two and five days after farrowing. Wilhelm (89) recommends a feed supplementation of sows during pregnancy with 50 IU vitamin E.kg^{-1} feed. According to Loudenslager *et al.* (53) selenium (1 ppm) and vitamin E (50 IU.kg^{-1}) supplementation of the diet of the sow maintains high vitamin E and Se levels in the colostrum and milk through the nursing period.

c) Bacteraemia

When bacteria invade an animal, their ability to grow and multiply depends on the availability of iron. Excess iron may stimulate the growth of pathogens in the blood (30). A certain amount of free iron is required for the growth and normal functioning of bacteria. For example, iron is involved in DNA synthesis within the bacterium. When the pig's iron blood levels are limited, cellular mitosis of the bacteria is severely curtailed (9). Therefore it is logical that polyarthritis occurs more commonly in piglets with an excess of iron (33).

Free iron ions catalyse the production of free radicals. The free radicals induce peroxidation of unsaturated fatty acids in the cell membranes. This leads to cell membrane damage, including those of macrophages. The iron overdose can in this way disturb macrophage function and diminish the defence ability of the organism (58). New investigations show that phagocytosis and the oxidative burst of granulocytes and monocytes may be impaired (50). Cardier *et al.* (10) have reported that the increase of spleen lipid peroxide levels was associated with a decreased lymphocyte proliferative response in iron overloaded rats. These results support the hypothesis that lipid peroxidation plays a role in the immunological abnormalities observed after iron overdose.

Vitamim E has been found to increase both cellular and humoral immunity in a variety of animal species, among them pigs (5, 56). After injection of iron overdose in form of iron dextran, there was an increased incidence of polyarthritis in suckling piglets. When vitamin E was given before iron injection, the incidence of polyarthritis in piglets decreased significantly (33).

The excess of iron-dextran can also block the body's defence mechanisms by overwhelming the phagocytic cells, which increases the likelihood of infection (46, 79, 89).

d) Calciphylaxis

A more rare form of toxicity is associated with calciphylaxis, the massive mobilization of calcium after injection of iron preparations. It is caused by the simultaneous application of high dose of vitamin D_3 and iron. It occurs within a few days after iron injection and is associated with the development of a hard swelling at injection sites and calcification in different organs, mainly in the lung, heart and kidneys. Affected piglets start to lose weight and polyuria, polydipsia, dyspnoe and coughing are observed (30, 89).

e) Lameness

When injecting into the gluteal muscles, *n. fibularis* and *n. tibialis* can be damaged. This can results in lameness (30, 64).

ORAL ADMINISTRATION

Oral iron supplementation in piglets has been a routine practice for many years, a wide range of products is available for this purpose. The preparations for oral application can be divided into following groups: iron-dextran, iron salts, chelates.

Iron-dextran

Apart from parenteral injection the macromolecule of iron dextran complex is used also for oral administration. Newborn animals will absorb macromolecules intact from the small intestine by pinocytosis (54, 55). The ability to take up bulk amounts of macromolecules by (un) specific pinocytosis provides the neonate with the passive immunity present in the mother's colostrum (65).

It has also been shown that not only immunoglobulins but also other macromolecules such as dextran are absorbed by the same mechanism. During the neonatal period epithelial cells of the small intestines have a system of apical tubules and vesicles and these cells have the capacity for pinocytosis. Vacuolation of the ileal epithelium is a necessary condition for uptake by pinocytosis (54, 55).

Martinsson and Jonsson (54) have found that pinocytosis of macromolecules is most pronounced in the lower half of the intestines. A smaller uptake is sometimes observed in the upper part, probably depending on individual variation or on an effect of colostrum intake.

Intestinal macromolecules uptake is present also *in utero* during the last two weeks of gestation but it is markedly less in the foetus than in the neonate (66). These observations suggest that ability to take up and transfer intact proteins from the epithelium into the circulation is a very specific process that develops close to term. This hypothesis is supported by the finding that the capacity for protein absorption is lower in newborn piglets delivered prematurely than in those born at full term (65).

In the pig the ability to absorb macromolecules ceases within the first day after birth, by a process known as intestinal closure (87). In piglets more than one day old no macromolecules are transmitted across the intestines. The mechanisms of this closure is not known, but several factors may be involved, such as endocrine factors, alterations of the absorptive cells, factors in colostrum or changing of the energy metabolism of the intestinal epithelial cells (54, 55).

In the pig, the low cortisol levels associated with preterm birth by elective caesarean section (65) are associated with severely reduced IgG uptake capacity. Nevertheless, it seems that the time of intestinal closure to macromolecule transport in the pig and other farm animals is influenced more by luminal factors than by endocrine factors such as glucocorticoids. Studies in foetal and neonatal pigs (66, 88) indicate that colostrum itself plays the most important role in the induction of intestinal closure (67).

Oral Fe-dextran dose is transferred into epithelial cells within about twenty hours from administration. The epithelial cells will stay functional for about seven days and transfer, within that period of time, most of the Fe-dextran to blood plasma or lymph (43). This was confirmed by investigations by and Thorén-Tolling and Jönsson (83) regarding the turnover time of the ileal epithelium in piglets. They found that time of complete replacement was about seven to ten days.

The Fe-dextran is transported in blood plasma as water soluble compound in unchanged form (8). Because of the large molecular size of iron-dextran complex, this complex is not excreted by the kidney (74). Only ca 1—2 % of Fe from iron dextran is presented as free Fe and can bind directly to transferinne. Most of the iron is taken up from blood plasma by cells of the reticulohistiocytic system, with the dextran component being decomposed by lysosomal enzymes and stored in the form of ferritin (41, 45).

According to Kolb *et al.* (46) the augmentation of the Fe-content in lymph nodes is induced by the ingestion of Fe-dextran by macrophages. The efficiency of the macrophages for immunological defence is temporarily reduced by Fe-dextran uptake (45, 46, 69). Kadiz *et al.* (39) have found that an overdose of iron-dextran renders piglets more susceptible to *Escherichia coli*-induced diarrhoeal disease.

No toxic effects after oral iron dextran administration has been reported so far. This could be explained by the relatively slower transfer of Fe-dextran from epithelial cells to blood plasma or lymphatics, which takes place within seven days (45).

According to Thorén-Tolling (82) the oral administration of iron-dextran does not disturb the intestinal absorption of IgG from colostrum in newborn piglets.

In order to ensure maximal efficiency the iron-dextran must be administered as soon as possible after birth. The administration should occur maximally up to six hours (30, 36) or eight hours (51) or ten hours (26) after birth. When it is given later, absorption decreases significantly because of intestinal shuts (36, 54, 87, 90).

Recommendations given on dosage and the number of applications differs among authors.

According to Gürtel *et al.* (29) and Kolb and Hoffmann (45) the administration of Fe-dextran (150 mg Fe) within eight hours of birth, followed by a second application of iron-dextran (150 mg) on the ninth day of life, results in a very good haemoglobin concentration in the blood. By single oral iron-dextran (200 mg Fe) administration, somewhat lower blood haemoglobin concentrations are obtained, but anaemia can be successfully prevented and a good intensity of growth can be achieved.

Glawischnig *et al.* (26) have reported that a single oral dose of 200 mg iron-dextran within ten hours of birth prevented piglets from becoming anaemic and was comparable to 200 mg iron-dextran given parenterally

According to Glawischnig *et al.* (26) and Lemacher and Bostedt (52) the single oral iron-dextran administration can be efficient provided that the piglets have free access to creep feed containing iron.

Another form for oral iron dextran preparation is iron microemulsion in which water soluble iron-dextran complex is bound to oil drops under the use of vegetable emulgator (36). This preparation has been evaluated by Iben (36) and Svoboda and Drábek (77) with different results. A single dose of 230 mg Fe was used in both trials.

Iben (36) concludes, that a single oral dose of Fe^{3+}-microemulsion is not sufficient in preventing iron deficiency anaemia and a second dose of iron *via* injection on the tenth day of life is necessary. However, haematological indices in his study were examined only until the tenth day of life of piglets, and no creep feed was offered to the piglets.

Svoboda and Drábek (77) have found that single oral administration of iron-microemulsion was efficient in preventing anaemia in piglets and it resulted in the growth intensity of piglets comparable to piglets given iron-dextran parenteraly. The piglets were offered prestarter (100 mg $Fe.kg^{-1}$) from day 3 to day 10 and starter (248 mg $Fe.kg^{-1}$) from day 10 to day 35.

Witschi and Heiritzi (90) also conclude that piglet anaemia can be prevented by the *per os* administration of iron microemulsion. A serum iron concentration above the critical level of 28 $\mu mol.l^{-1}$ could be maintained over a 21-day period with a double administration of 115 mg Fe as microemulsion. The first iron dose was given 8—12 h p.p. and the second on day 12 p.p. The piglets receiving their iron according to this method, upon reaching day 28 p.p., achieved both the highest weight as well as the highest daily weight gain when compared to piglets being treated according to other methods.

Iron salts

The iron ions are released from iron salts in the intestine. Examples of iron salts are iron-sulfate, iron-fumarate, iron-lactate. Those preparations must contain Fe^{2+}. Fe^{3+} ions form highmolecular, polynuclear iron hydroxide in an acid environment, which is almost undissociable and is rapidly excreted in the faeces (74). Apart from this, the presence of complex building substances derived from feed such as phytate, pyrophosfate and citrate worsens iron resorption. Fe^{3+} ions form much stronger complexes than Fe^{2+} ions. Bivalent iron Fe^{2+} is absorbed up to 16 times better than trivalent iron Fe^{3+}. Therefore only preparations with bivalent iron can be recommended for oral iron therapy (15).

Orally administered iron is absorbed by mucosa in the cranial part of the small intestine. Enterocytes bring iron to the blood where it binds with transferrin (35). Maximal absorption occurs in the duodenum and upper jejunum under optimum pH conditions. In general, a lower pH, favors Fe^{2+} state and iron absorption, whereas a neutral or alkaline pH favors the Fe^{3+} state and decreases iron absorption (57). Peroral Fe is available for haemoglobin synthesis sooner after administration than injected Fe-dextran (24). A part of the absorbed iron is retained in enterocytes in the form of reserve mucosal ferritin. Iron present in spontaneously desquamating enterocytes can be reabsorbed from intestinal contents (68).

These facts emphasize the extraordinary importance of normal intestinal functions in the utilisation of orally administered iron. This does not pertain only to the topical state at the time of treatment, but also to the subsequent four to seven days which are the period for the physiological turnover of enterocytes. During this period, iron bound in intestinal ferritin can be utilised, or lost if diarrhoea develops. These facts must be considered when deciding whether and when oral administration of an iron-containing preparation is appropriate (47).

If an excess of iron occurs in the mucosal cell, ferritin synthesis is stimulated, and iron is deposited in ferritin to prevent oxidative damage to the cell from ionic iron. The "mucosal blockage" of iron absorption has limited capacity, because higher doses of iron can overcome the blocking mechanism. In domestic animals, iron toxicity can occur when they are exposed to high iron levels in feed or water (72).

Before the oral application of iron the piglets should be allowed to suckle colostrum several times (47). Le Dividich *et al.* (49) have demonstrated that colostrum intake induces an almost immediate increase in the weight of the small intestine and changes in its structure and functions. The highest relative gain occurred during the first six hours after birth and the major part of this gain comes to the intestinal mucosa. Longitudinal and lateral growth of intestinal villi increases the absorption surface twofold.

A comparative study of iron availability from commercial preparations of $FeSO_4$, ferrous gluconate, ferrous fumarate, and a polysaccharide-iron complex using *in vitro* digestion cell culture model was carried out by Glahn *et al.* (25). Significantly more iron was taken up from $FeSO_4$, ferrous gluconate and ferrous fumarate than the polysaccharide-iron complex. Fe^{2+}-fumarate based products for oral application have been used successfully in human medicine (12, 48).

Gleed and Sansom (27, 28) have repeatedly reported that feeding sows a diet enriched with 2000 mg $iron.kg^{-1}$ of feed as iron-sulfate satisfactorily protected their piglets from anaemia without the need for injection of iron dextran. The piglets grew as well as piglets reared conventionally which had received 200 mg iron as iron-dextran i.m. The sows were housed in solid-floored farrowing pens of approximately 3 × 2.5 m and were able to move over the whole area. Creep feed (450 mg $iron.kg^{-1}$) was offered to the piglets from 14 days of

age. The piglets were observed to eat the faeces and the sows teats often became contaminated with faeces and were subsequently cleaned by the suckling piglets. The piglets ingested on average twenty grams of the sow's faeces daily.

This method was confirmed by K o l b *et al.* (44). According to these authors an effective prophylaxis against anaemia of piglets could be achieved by adding twenty grams of iron-sulfate (4 g Fe) to fodder concentrates which were fed to sows from two days before and 28 days after parturition. In this way a high iron content in faeces was achieved, that was in a relatively large amount consumed by piglets. The piglets of these sows received an additional per-kilogram ration of 2.5 g of iron-sulfate.

K o l b *et al*. (44) suggest that by sufficient transferrine saturation with iron, only a small amount of iron is transferred from enterocytes to blood plasma. This regulative mechanism works also through a high uptake of iron salts. The very high concentration of iron in faeces makes it possible that piglets can receive enough iron by eating small amounts of faeces.

The efficiency of oral Fe^{2+}-fumarate supplementation in preventing piglet anaemia under experimental conditions has been evaluated by K o t r b á č e k (47). In his study the Fe^{2+}-fumarate paste was administered to day-old piglets and haematological values were measured until 21 days of age. The effect of oral administration of 100 mg fumarate-bound iron on haematological indexes in this period was equal to that of the parenteral administration of 200 mg Fe^{3+} and even stronger in some indexes in the second week of life. In a study conducted by S v o b o d a and D r á b e k (78) a repeated dose of 200 mg Fe as iron-fumarate on day 6 and 11 was necessary to achieve comparable haematological values to those obtained by i.m. administration of iron-dextran. This could be explained by the fact that intestinal disorders, which may develop in the postnatal period, can effect the utilization of orally administered iron.

The efficiency of iron-lactate in preventing iron deficiency of piglets under experimental conditions was reported by K o t r b á č e k (47).

Oral administration of iron can be efficient providing the gastrointestinal system is normal (64, 81). For instance A c k e r m a n *et al*. (1) have demonstrated a marked detrimental effect of transmissible gastroenteritis on absorption of iron from the intestinal tract.

Chelates

Another possibility of iron supplementation to pigs is aminoacid-chelated iron. Iron chelates due to their high complex stability remain intact in the gastrointestinal tract and are absorbed as a complex. The iron chelates are very well absorbed, but the disadvantage is that part of the absorbed iron-chelates is excreted by the kidneys and is lost for further utilization (14, 74).

The iron chelates were used as an additional source of iron for voluntary uptake by piglets after single iron administration (21, 38). The iron chelates offered to suckling piglets in drinking water as a sole source of iron were not efficient (22).

Many attempts to prevent the development of anaemia in piglets by treating pregnant sows with iron preparations have failed. Some promising results have been achieved with amino acid-chelated iron, although the effects have been too small to prevent anaemia during the pre-weaning period (2, 3). Amino acid-chelated iron is thought to be absorbed as amino acid-complex or as small peptides. A higher absorption rate is therefore achieved (3), and is also believed to cross the placental barrier more readilly than other iron compounds (3). In a recent study conducted by E g e l i *et al*. (22) sows were given 300 mg iron daily as amino acid-chelated iron during the last three weeks of gestation. A slight increase in haemoglobin concentration and red blood cell count in the piglets from the iron-treated sows was found compared with controls, but this was considered to be of no practical importance (22).

Forms of oral application

Several methods of oral application of iron to suckling pigs have been tried. Some of them are: giving suckling pigs access to parasite-free soil in the pens, painting a viscous paste on teats and mammary glands of the lactating sow, spraying an aqueous solution of iron compounds on the mammary glands of the sow and administering iron solution or iron tablets orally. The effectiveness of these attempts in the treatment and prophylaxis of anaemia in swine is controversial (73).

Because it is considered that the voluntary consumption by piglets of such products (oral preparations) during the first week of life will be inadequate, the practice of administering an oral dose directly into the mouth in newborn piglets has been employed (20).

Iron-containing paste are used commonly. Such product can contain also a probiotic assuring the colonisation of the digestive tract by beneficial lactacidogenic bacteria and can be used as a source of selected minerals and vitamins (47).

Another possibility for oral supplementation with iron is the voluntary uptake of iron-containing preparations offered to piglets *ad libitum*. One of the alternatives is the enrichment of prestarter diets with readily absorbable forms of iron. The efficacy of such supplementation has been found insufficient because the consumption of prestarter in the first two weeks after birth is low (18).

It is generally agreed that application of such preparations in drinking water is not recommended, because as long as piglets suckle they are not really interested in other water sources (20, 38, 91).

However there are a few reports, that voluntary iron uptake can be successful. B o l l w a h n and S c h u l z e - S t e i n e n (7), Z i m m e r m a n n (91), J ö r g e n s e n and B r u n (38) have managed to prevent piglet iron deficiency anaemia by offering piglets solid iron preparations (powder) with a high iron content from the second day of life. The iron in these preparations was present at a very high concentration in the form of iron-fumarate (7), and combination of iron-sulfate and iron-fumarate (38, 91). It is obvious that the iron content in such preparations must be very high, however there are so far no objective data evaluating the influence of sensoriale

attractivity (smell, taste) of such preparations on voluntary uptake by piglets.

CONCLUSION

Single parenteral or single oral administration can be sufficient in preventing piglet anaemia provided they have free access to creep feed, which they start to eat intensively mostly at the age of three weeks (26, 52).

The iron injection is a more reliable method than oral administration. Misapplication of an oral preparation can occur and some of the piglets may not always swallow the oral preparation properly (91).

On day 7 or 8 of age haemoglobin concentrations obtained by early postnatal iron-dextran oral administration are significantly higher than the values after the usual iron-dextran injection on the third day of life (36, 51, 77). However according to Svoboda and Drábek (77) and Egeli and Framstad (20) the somewhat lower haemoglobin levels on day 7 do not cause lower weight gains. In a study conducted by Lemacher and Bostedt (52) no other significant differences have been seen between the oral or parenteral iron dextran treated groups reared on an iron-containing floor (metal slats). Although in the farrowing on plastic crates the piglets supplemented orally with iron dextran showed a decrease of haemoglobin level starting at day 14. At day 24 the values of haemoglobin were significantly lower than after an iron injection.

In intervals of 3, 6, and 9.5 hours after oral iron-dextran administration the highest Fe concentration is found in the intestinal lymph nodes. After an intramuscular injection the Fe content of the inguinal and iliac lymph nodes increases very much (45). Seven days after iron-dextran injection the distribution of stainable iron in lymph nodes, liver and spleen is comparable to that after oral administration (83). The augmentation of Fe content in the lymph nodes is induced by the ingestion of Fe-dextran by macrophages. In this way the capacity of the immune system is apparently reduced for some time (46).

Further studies are needed to show, if there are differences between the temporary lowering of phagocytic activity after oral and i.m. iron-dextran administration. A dose of 200 mg Fe administered orally to pigs shortly after birth has a greater adverse effect on their susceptibility to enterotoxic colibacillosis than does the same dose given i.m. at the same time interval after birth (39). It seems that oral iron-dextran administration has a more significant impact on intestinal lymph nodes than i.m. injection.

No toxic effects, that may occur with iron-dextran injection, have been reported after oral iron-dextran administration so far. This could be explained by the relatively slower transfer of Fe-dextran from epithelial cells to blood plasma or lymphatics, which takes place within seven days (45).

The presence of diarrhoea and intestinal infection in a herd prevents absorption of orally administered iron and treatment by injection is recommended in this instance (1, 64, 81).

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