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Investigation of sodium metabolism in the ruminants, in vitro and in vivo studies with 24Na isotope, after different intake of potassium and water, (part of a coordinated programe on water requirements of tropical herbivores based on measurements with tritiated water)

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Investigation of electrolyte and water metabolism in the ruminats after different intake of sodium and water[x]

by

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The water and electrolyte content of the rumen which gives the 15 % of the water space of the body has an important role in the regulation of the water and electrolyte metabolism of the sheep. The diuresis is very important in the standardisation of the volume of the body-water space but in the ruminant the saliva secretion is also a significant-factor in the water and electrolyte metabolism of the body.

In our experiments the mechanism of the regulation of the water and electrolyte metabolism was examined in two points of view. In one hand the range of the ion transport was determined *in vitro* in the sheep's erythrocytes of low and high K^+ content. On the other hand in 3 in *vivo* experiments the function of water and salt metabolism was examined during saline overcharge by animals supplied with different quantity of saline and water and the alterations of Na^+ and K^+ concentrations of plasma and saliva, moreover the saliva secretion and the diuresis rates were studied.

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In vitro experiments:

In our former experiments it was examined how the different factors /haematocrite values and Na^+ concentrations/ influenced the function of the Na-pump in sheep's erythrocytes. On the basis of our results it can be established, that the different haematocrites values have no effect on the active transport of Na^+ from the erythrocytes to the solution. In the case of the solution with high Na^+ concentration the Na^+ transport of the suspensions increases. The Na-pump takes a larger amount of Na^+ to the solution and this is the consequence of the higher Na^+ content of the erythrocytes. In our experiments the mean Na^+ concentration of the erythrocytes was 80-90 /uaeq/ml in a solution with normal Na^+ concentration. The K_E value became 3,8%. This means that the quantity of the Na-ions coming from 1 ml erythrocytes per hour in average 3,17 /uaeq.

It is well-known that sheep is a species showing low red cell K^+ /LK/ and high red cell K^+ /HK/ dimorphism. In our experiments of the last year we studied the Na^+-pump in red cells with low and high K^+ content. The red cells being used in the experiments were taken from Hungarien Merino-sheep. The K^+ concentration of the HK red cells was seven times higher than that of the LK red cells /85, respectively 12 /uaeq/ml/, but their Na^+ concentration was two and a half times lower /35 respectively 90 /uaeq/ml/. The erythrocytes being filled up with ^{24}Na isotopes were suspended in every case into a solution of 5,9 /uaeq/ml K^+ and 146 /uaeq/ml Na^+ concentration and we have mesured hourly the decrease of their radioactivity during 7 hours. We calculated a constant /α/, which is characteristic to the speed of change of the $^{24}Na^+$ between the red cells and the solution. The speed coefficientes /β_E and β_P/ of Na^+-transport into and out of the red cells were calculated from the α-constant. We counted the quantity of Na^+-s, partly transported into the erythrocytes and out of there /$\overleftrightarrow{Na^+}$/ in unit volume suspension, partly coming out from unit volume of the red cells /$\overrightarrow{Na^+_E}$/ during one hour.

The following conclusions can be drawn, when studied the characteristic data to the Na^+-transport of LK and HK red cells. The α-value which is characteristic to the speed of change of the $^{24}Na^+$ between the erythrocites and the solution is five and a half times higher in the case of HK than of LK red cells. The speed coefficient of the active transport of Na^+ from the red cells /β_E/ is six and a half times higher the speed coefficient of the passiv transport into the red cells /β_P/ is three and a half times higher in the case of HK erythrocytes.

The quantity of Na^+ flowing hourly into and out of the red cells /$\overleftrightarrow{Na^+}$/ being in 1 ml suspension is significantly higher in case of HK than of LK red cells /3 respectivly 1 μaeq/ml/. Similar difference can be seen with the quantities of Na^+ /$\overrightarrow{Na^+_E}$/ coming out hourly from 1 ml red cells by the effect of the Na-pump. This is two and a half times higher in case of the HK red cells than that of LK red cells / 8, respectivly 3 μaeq/ml/.

On the basis of the above-mentioned data it can be said that the Na^+-transport of the HK red cells is significantly greater than that of the LK red cells.

<u>In vivo experiments</u>

In our former experiments we have studied by sheep with ruminal and salivary fistulas, and urinary bladder catheter, the effects of water and NaCl given in excess amounts to the animals. The quantity of saliva and urine production /diuresis/ as well as the elimination Na^{+} were determined. Changes of the extracellular space /EC/ during sodium and water overcharge were measured by means of $^{24}Na^{+}$.

We have found that the mechanism of saliva secretion depended during the first two hours on the actual osmotic conditions of the rumen and was unrelated to the conditions of the EC. If fluid supply is satisfactory, saliva secretion depends on the ruminal electrolyte concentration. If the EC space is reduced, the absorption of electrolyte quantity decrease and the saliva secretion rate adapts onself to the fluid deficiency. It seems that primary stimuli of this adaptation originating from the chemo- /or osmo-/ receptors of the rumen wall and conducted to the central nervous system via a reflex arch and gets to the salivary gland and this alters its secretion. Thus the amount of saliva secretion appears to depend directly on the electrolyte concentration of the rumen fluids.

Studying further the electrolite- and water metabolism of ruminants we proved. in our experiments, that the Na^{+} and K^{+} concentrations of saliva give good information about the electrolyte and water transport of the ruminant organism. On the base of the above mentioned connection we studied also the saliva secretion, the diurezis and the Na^{+} and K^{+} excretion of sheep which had participated in different quantity of saline supply before getting saline oversharge. In our experiments we determined to obtain more exact data of the connections between the saliva secretion and the diuresis, and of the mechanism of the Na^{+} and K^{+} transport.

Our last experiments were done on 16 sheep which were provided with rumen fistula and catheters in the salivary tube, urether and v. jugularis. Before the experiments the animals had be fed by different saline content fodder for 4 weeks.

In the experiment the retaining samples from the rumen, blood saliva and urine were done always at the same time. The salivary and urinary samples were collected into fractions of 3 or 10 ml, equipped with automatic register to measure quantity of saliva and of urine being produced per minute.

300 ml water with 200 μc $^{24}Na^{+}$ were given ir. to the animals. In addition, the experimental animals got NaCl 1 g/kg bw. in water of the same quantity ir.

Before the saline overcharge the normal values of the quantities of salivary secretion and diuresis have been registrated during 40 minutes. We measured the quantities of salivary secretion and of diuresis per minute, the Na^{+} and K^{+} concentrations of the rumen content, plasma, saliva and urine. We counted the quantities of Na^{+} and K^{+} secreted in the saliva end excreted in the urine, and the Na^{+}/K^{+} quotient of saliva and we measured the cpm-number in the samples.

In the <u>first part</u> of our experiments it was studied how much is the Na^{+} and K^{+} concentrations of the plasma and the saliva in different saline /NaCl/ supply and how active the Na^{+} secretion of salivary gland is in different saline supply, followed by saline overcharge /ir./. Namely it was studied that in these conditions what kind of cpm number was reached by $^{24}Na^{+}$ absorbtion in the plasma and what is the time during the cpm-number of saliva exceeds the concentration of the plasma.

The averages Na^{+} concentrations of the plasma in each experimental groups which had got different saline supply and also in the control group were between 140-150 maeq/l; the K^{+} concentrations are among 4,3-5,8 maeq/l /<u>Table</u> 1./.

It was seen that Na^+ concentration of the saliva in case of plentiful saline supply /A/ was 182 maeq/l, the concentratio of K^+ was 4,8 maeq/l.

The Na^+ concentration of the saliva of sheep getting sufficient /normal/ saline supply /B/ was 155, the K^+ 5,4 maeq/l. The Na^+ concentration of the saliva by deficient saline supply /C/ was 116, the K^+ 12,6 maeq/l.

The Na^+/K^+ quotients are the same in case of control animals /D/ and in animals getting plentiful saline supply /A/. This quotient has an average value in case of the sufficiently fed animals /28;B/ and has a very low value /9/ in case of deficiently fed sheep /C/.

After the NaCl overcharge in group A the $^{24}Na^+$ cpm number of the saliva extended the plasma after 11, by group B and C 40 minutes. In the case of the control animals /D/ the cpm number of the $^{24}Na^+$ in the saliva became equal or higher to the plasma cpm number after 21 minutes.

It can be well observed that the global saline content of the sheep's organism respectively the quantity of NaCl intake by the fodder can charge the quantity of Na^+ being secreted by the saliva. As higher is the NaCl intake, increase the Na^+ secretion in the saliva. The concentration of K^+ does not change usually , but in case of deficient saline supply it become doubled in the saliva. The time during the cpm-number of $^{24}Na^+$ become higher in the saliva than in the plasma was shortest by the animals with plentiful saline supply and by the control groups; deficient animals was longer.

In the second part of our experiments the parameters related to the water and electrolyte metabolism were measured. First of all it was studied how the quantity of electrolytes secreted in the saliva and excreted in the urine before and after ir. saline overcharge? These were observed in sheep getting different saline supply /Table 2./.

In the control group /D/ the secretion rate of the saliva was 0,86 ml/minutes; the quantity of the secreted Na^+ was 152 and of K^+ was 3,7 /uaeq/minutes. The diuresis was 1,07 ml/minutes the quantity of Na^+ excreted by the urine per minutes was 68, and of K^+ 296 /uaeq.

In the sheep with plentiful saline supply /A/ the quantity of the saliva being secreted per minute was 0,81 ml before; and was 0,88 ml after the saline overcharge. the quantity of the secreted Na^+ was 161 before, and 159 /uaeq/minutes after the overcharge. The quantity of the secreted K^+ was 3,8 before and 3,9 /uaeq/minutes after the overcharge. The rate of the diuresis increased from 0,79 to 124 ml/minutes. The quantity of Na^+ excreted by the urine was 184 /uaeq/minutes after the overcharge. The same of K^+ was 305 /uaeq/minutes. These values were lower /144 respectivly 243/ before the saline overcharge.

The salivary production rate of sheep by sufficient saline supply /B/ decreased from 0,70 to 0,45 ml/minutes by the saline overcharge. The quantity of the secreted Na^+ decreased from 97 to 66 /uaeq/minutes and the K^+ decreased from 3,2 to 2,8 /uaeq/minutes. The diuresis increased from 0,66 ml to 0,84 ml/minutes and the Na^+ quantity excreted by the urine increased from 36 to 140 /uaeq/minutes the K^+ increased from 42 to 104 /uaeq/minutes.

In case of the group with deficient saline supply /C/ salivary secretion rate decreased by the effect of saline overcharge from 0,56 to 0,40 ml/minutes; the Na^+ secretion also decreased from 50 to 43 /uaeq/minutes; the quantity of K^+ decreased from 2,6 to 2,1 /uaeq/minutes. In the contrary, the diuresis increased from 0,39 to 0,56 ml/minutes; also the excreted Na^+ quantity from 15 to 87 /uaeq/minutes. The quantity of K^+ excreted by the urine increased only from 61 to 113 /uaeq/minutes.

From our experiments it can be establich, that the salivary secretion and the diuresis change reciprocally in the regulation of the water and electrolyte metabolism, mainly by sufficient and deficient saline supply and after NaCl and water overcharge. When overcharged with saline the extra quantities of Na^+ are excreted only by the urine, and in this case the Na^+ concentration and secretion the saliva decreases. When the NaCl intake with the food is on a low level, the Na^+ and K^+ quantities secreted with the saliva and urine decrease. The overcharge with NaCl increases in all cases the excreted quantities of Na^+ and K^+ in the urine but not in the saliva.

In the third series of experiments we studied the effect of a sudden saline overcharge on animals with watersupply ad libitum and on thirsting animals. We compared the results of these experiments with the values of experiments with water overcharge on animals supplied with water ad libitum. In case of saline overcharge after thirsting the dosis of NaCl was 0,75 g, after supplying wiht water ad libitum 1,0 g/kg bw. The NaCl was in all cases dissolved in 300 ml water and it was given it. The amount of water overcharge was 80 ml/kg bw.

The results of our experiments gave the answer, how the organism of sheep reacts upon the sudden saline overcharge without water withdrawal and after thisrt? We also got the answer, how the water and electrolyte metabolism changes by the effect of a strong water overcharge? In our experiments we measured the Na^+ and K^+ concentrations of the rumen content and the changes of the water content in the rumen by isotope dilution method. The Na^+ and K^+ concentratic of the blood plasma as well as the time of the appearing of $^{24}Na^+$ isotope in the saliva were studied after the different treatments. In addition we studied changes of the Na^+ and K^+ concentrations in the saliva and in the urine, furthermore the changes of the salivary secretion rate and diuresis. We calculated the quantities of electrolytes excreted per minutes. The values presented in the tables are representative ones, and they can be regarded as the average of the results got during the experimental period of 360 minutes. We don't present values of statistical calculations, because only those resultes were evaluated biologically which showed great differences in change.

It can be seen in the table 3. that the Na^+ concentration and the quantity of the rumen fluid it was increased by saline overcharge. After thirst of 48 hours the K^+ and water contents of the rumen fluid decrease, but the Na^+ concnetration increases. The water overcharge reduces the Na^+ and K^+ concnetrations of the rumen fluid and increases the water content of it.

On the table 4. you can observe, that the NaCl overcharge does not increase significantly the Na^+ content of the plasma, but decreases the K^+ concentration. Thirst increases the Na^+ concentration of the plasma, but does not influence the K^+ content. The saline overcharge after thirst have only a little effect on the electrolyte concentration of the plasma. On the other hand the water overcharge decreases both the Na^+ and the K^+ content in the plasma. It also can be seen, that both the saline and water overcharges increase the rate of the secretion of Na^+ from the plasma to the saliva.

On the table 5. you can see the changes of the Na^+ and K^+ concentrations in the saliva and in the urine, the changes of salivary secretion and diuresis and the changes of the quantity of Na^+ and K^+ excreted with them per minute. The saline overcharge does not influence the Na^+ and K^+ concentrations of the saliva, but it increases the Na^+ concentration and diminishes the K^+ concentration in the urine. The rate of salivary secretion decreases, but the diuresis increases. The quantity of the Na^+ and K^+ secreted per minute with the saliva decreases. In the urine the excreted Na^+ becomes six times as much as it has been, and the excreted K^+ scarcely changes.

In the case of thirst the Na^+ and K^+ concentrations of the saliva and of the urine change only a little. The salivary secretion was doubled and the diuresis was reduced to its half. The Na^+ secretion with the saliva increased highly but with the urine only a little. The secretion of the K^+ was approximately the same. The saline overcharge after thirst did not influence the Na^+ and K^+ concentrations of the saliva, while it increased significantly the Na^+ but not the K^+ content of the urine. On the contrary both the secretion of the saliva and the diuresis decreased. The salivary secretion of Na^+ and K^+ decreased with one third. The urinary secretion of Na^+ changed scarcely, but the secretion of K^+ decreased strongly.

In case of water overcharge the Na^+ concentration of the saliva did not change, but that of the urine increased. The K^+ concentration as well as the secretion of the saliva and urine remained on the same level. The salivary secretion of Na^+ and K^+ did not change. The urinary secretion of Na^+ became threefold, that of the K^+ double.

Compairing the results we got in the rumen with those we got in the saliva and urine it can be established, that the increase of the electrolyte /Na^+/ concentration in the rumen content - in consequence of saline overcharge, - caused a higher Na^+ and a lower K^+ concentration in the urine. In these cases the rate of salivary secretion decreased and that of the diuresis increased. In this way the excretion of Na^+ with the urine became six times greater but the secretion of Na^+ with the saliva decreased.

The thirst caused the increase of the Na^+ concentration in the rumen content; it redoubled the rate of salivary secretion and reduced the diuresis to its half. The secretion and excretion of Na^+ increased. In case of thirst more fluid and Na^+ get back to the rumen by the saliva, but less of them leave the organism by the urine.

The intake of NaCl after thirst increases the Na^+ concentration of the rumen content. This results in the increase of the Na^+ concentration of the urine. The saliva and the urine react ot this with the decrease of the secretion rate and therefor the degree of the Na^+ excretion with the urine does not change.

The water overcharge increases the quantity of the rumen content and reduces the Na^+ and K^+ concentrations in it. At the same time the Na^+ and K^+ concentration increases in the urine and their excretion increaes too.

Compairing our experimental results again we can establish, that the intake of electrolytes and water, or the thirst did not change significantly the isotonia and isoionia of the blood, but they had a quick and significant effect on the electrlyte concentration and on the secretion of saliva and diuresis. The NaCl overcharge generally increases the Na^+ concentration of the urine as well as the diuresis and it decreases the K^+ concentration of the urine and the secretion of the saliva. The thirst increases the secretion of the saliva only /doubly/, but it decreases the diuresis. The Na^+ secretion by the saliva and the Na^+ excretion by the urine increase by the effect of thirst. The saline overcharge after thirst decreases both the salivary secretion and the diuresis as well as the Na^+ secretion with the saliva. The water overcharge increases the Na^+ concnetration of the urine by the effect of the increase of the rumen fluid and the excretion of Na^+ increases threefold with the urine.

We can draw the conclusion from the results of our experiments, that the change of the osmotic concentration of the rumen /mainly the water and electrolyte content/ influences the water and electrolyte metabolism of the organism quickly and significantly. It is well-known, that the acid-base-balance of the organism is closly related to the water and electrolyte metabolism, so we intend to turn our attention on this problem in the future.

Table 1.

The effect of saline supplied with different quantity in the feeds

Sign	Saline-supply	NaCl percentages feeds	Plasma Na^+	Plasma K^+	Saliva Na^+	Saliva K^+	Saliva Na^+/K^+	Increase of saliva Na^+ concentration[xx] minutes
			maeq/l		maeq/l			
A	plentiful	0,60	142	4,7	182	4,8	38	11[x]
B	sufficient	0,45	141	4,8	155	5,4	28	42[x]
C	deficient	0,20	149	4,3	116	12,6	9	40[x]
D	control	ad. lib.	144	5,8	176	4,4	40	21

x NaCl overcharge 1 g/kg bw., ir.

xx The time during the cpm-number of $^{24}Na^+$ become higher in the saliva than in the plasma

Table 2.

The effect of saline overcharge[x] in animals supplied with different quantity of saline in the feeds

Sign	Salivary secretion		Na^+ secreted in saliva		K^+ secreted in saliva		Diuresis		Na^+ excreted in urine		K^+ excreted in urine	
	before	after	before	after	before	after	before	after	before	after	before	after
	overcharge ml/minute		overcharge, uaeq/minute				overcharge ml/minute		overcharge, uaeq/minute			
A^x	0,81	0,68	161	159	3,8	3,9	0,79	1,24	44	184	243	305
B^x	0,70	0,45	97	66	3,2	2,8	0,66	0,84	36	140	42	104
C^x	0,56	0,40	50	43	2,6	2,1	0,39	0,56	15	87	61	113
D	0,86		152		3,7		1,07		68		296	

x NaCl overcharge 1 g/kg bw., ir.

Table 3.

The change of the $Na^{\pm}$ and $K^{\pm}$ and watercontent in the rumen

Watersupply, overcharge	Na^+	K^+	Watercontent litre
	maeq/1		
Ad libitum	92	29	1,7-2,4
NaCl overcharge /1,0 g/kg bw/	241	28	2,7
Thirst /48 hours/	164	20	0,7
NaCl overcharge /0,75 g/kg bw/	431	18	1,0
Ad libitum	100	26	1,8
Water overcharge /80 ml/kg bw./	60	16	5,0

Table 4.

The Na^+ and K^+ contents of the blood plasma

Watersupply, overcharge	Na^+	K^+	Increase/minute/ of the Na^+ concentration in the saliva[x]
	maeq/l		
Ad libitum	143	4,4	13
NaCl overcharge /1,0 g/kg bw/	150	3,7	32
Thirst /48 hours/	156	4,1	--
NaCl overcharge /0,75 g/kg bw/	158	4,1	14
Ad libitum	153	4,3	11
Water overcharge /80 ml/kg bw./	148	3,7	38

x The time during which the cpm-number /$^{24}Na^+$/ in the saliva becomes higher than in the plasma

Table 5.

The change of Na^{+} and K^{+} content in the saliva and in the urine, the change of their secretion or excretion and the change of the salivary secretion and of the diuresis

Watersupply, overcharge	Material	Na^{+}	K^{+}	Secretion diuresis ml/min	Na^{+}	K^{+}
		maeq/l			secreted µaeq/min	
Ad libitum	Saliva	176	5,1	0,86	152	4,4
	Urine	107	218	1,07	56	184
NaCl overcharge /1,0 g/kg bw/	Saliva	181	4,7	0,68	122	3,2
	Urine	211	62	1,42	361	203
Thirst /48 hours/	Saliva	179	4,6	1,41	255	6,2
	Urine	167	284	0,51	75	141
NaCl overcharge /0,75 g/kg bw/	Saliva	189	4,6	0,84	157	3,7
	Urine	284	291	0,35	72	105
Ad libitum	Saliva	173	5,0	0,57	98	2,8
	Urine	16	47	4,90	70	74
Water overcharge /80 ml/kg bw./	Saliva	169	4,2	0,59	100	2,3
	Urine	50	71	4,90	212	167