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Pathophysiological and Functional Aspects of the Megacolon-Syndrome of Homozygous Spotted Rabbits

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With 6 tables

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Summary

The Megacolon-Syndrome is a hereditary disease of homozygous spotted rabbits (En En). Investigations have been performed on some special traits related to functional aspects of the gut in comparison to vital heterozygous spotted rabbits (En en). It was found that En En rabbits showed significantly reduced sodium absorption rates across the wall of the cecum. Consequently, the dry matter content of the ingesta was reduced at this location, whereas the content of the ashes was increased. These results indicate that a further important pathogenetic aspect of this hereditary disease is an undue liquification of ingesta in proximal parts of the large intestine. Severe clinical problems, however, resulted from obstipation. This is concluded to be a late complication due to and modified by different stressors of endogenic and exogenic origin. Thus there are some indications that an early site of spot-gene related effects might be the small intestine. This segment of the bowel was shorter and had an increased dry matter proportion of its wall when compared with heterozygous spotted rabbits. But a decreased proportion of dry matter within the wall of the large intestine was found. The latter could be an effect of the hypothyreotic state of metabolism in En En rabbits.

Introduction

In recent years investigations have been made on subvital effects in a special phenotype of spotted rabbits. These animals connatally have reduced areas of pigmented skin resp. fur ('White Spot' rabbits, so called 'Chaplins') and their mortality is known to be increased during growth, especially in weaner rabbits, as has been described previously for purebred homozygous spotted genotypes (En En) (NACHTSHEIM, 1943; NIEHAUS, 1952). In contrast, heterozygous spotted ones (En en) are vital and the area of pigmentation of the fur is more extended. These standard animals could be used for show purposes when purebred only, whereas vital self-coloured rabbits (en en) and homozygous spotted rabbits from fancy stocks do not fit the rigid breed standards and, therefore, are often killed directly after birth; this is of relevance due to the German legislation for animal protection (WEGNER, 1991; WEGNER, 1995). In spite of the results of previous investigations, the overall mortality in En En rabbits

still was not elevated when outbred and reared under the local standardized conditions (GÖRSE, 1994).

However, nearly no information had been available with respect to the causes of this subvitality of En En rabbits and the aetiopathogenesis remained open still. However, recent research has pointed out that the main symptom of this hereditary predisposition consists in a megacolon which is characterized by increased absolute and relative weights of the gut in a filled and emptied condition (WIEBERNEIT et al., 1991). The trigger point, i.e. the onset of this maldevelopment, has not yet been identified but a hypoganglionotic state especially of the Plexus myentericus in the distal part of the colon (GERLITZ et al., 1993) and a moderate hypothyreosis (FLEMMING et al., 1994) are suspected to be important factors in the pathogenesis of this disease. However, the pituitary gland and the spinal cord seemed not to be involved primarily, although increased absolute and relative weights of the adrenals were found in En En rabbits (GÖRSE, 1994). Furthermore, severe clinical problems of this disease resulting from obstipation especially within the cecum went hand in hand with an over-proportional hypertrophy/-plasia of the adrenals (MAHDI et al., 1992). It was stated that a stress-related dehydration of the ingesta causes such features.

It was the aim of the present work to get further information about traits with possible relevance for the disease proneness of the En En genotype. Therefore, electrophysiological parameters of isolated cecal walls from spotted rabbits of different genotypes were studied. However, no data exist in the literature about ion transport capacities of rabbits with En En evoked Megacolon-Syndrome. In addition, the digestive tube was probed for pH-values at different locations. Furthermore, the content of dry matter of the ingesta and the content of dry matter of the gut wall was determined respectively. Finally, the ingesta were measured for the proportion of the ashes of its dry matter. All analyses have been performed in comparison to vital heterozygous En en rabbits.

Material and Methods

Twenty-six genetically defined animals were investigated belonging to both spotting-genotypes and being heterozygous (Cc) or homozygous for albinism (cc). Albinism was introduced from 'New Zealand White' rabbits (NZW) to test the hypothesis whether epistasis could suppress the pathological effects of the En-gene or a closely linked defective (WIEBERNEIT and WEGNER, 1995). For this purpose the litter mates were bred to be completely of homozygous or completely of heterozygous spotting types but 50 % could be albinotic in each litter. Thus, homozygous and heterozygous spotted animals could not be litter mates. The animals represented the final generation of a breeding experiment from two parental lines which were inbred (F_i = 0.1875 and 0.125) after three generations of purebreeding ES (English Spot breed) resp. DRS (German Giant Spot breed) and breeding two generations of two line crosses and two generations of three line crosses. All the generations were not inbred. Since the foundation of the stock, fattening performance and morphometrical analysis of the organs of all slaughtered animals were tested as a routine (WIEBERNEIT et al., 1991). In addition, special organs with possible relevance to the pathogenesis of the 'Megacolon-Syndrome' were analysed histometrically and statistical tests confirmed the conclusions given in the introduction.

For illustration of the spot-gene related influence parameters typifying the poor development and impaction of the bowel of En En—genotypes in comparison to heterozygous ones are given from all test animals of this sample (Table 1).

Immediately after slaughtering (stunning by a shot of a bolt, draining of the blood from stab of the throat), the entire intestine was removed. In the intestinal content of 12 animals (six En En and six En en) of group A (F_i = 0.1875) belonging to four different litters pH-values were measured at five different locations. Ingesta were collected from the caput ceci, the apex ceci and the distal colon and analysed for dry matter content. In addition, small pieces of the wall taken from eight different locations were analysed for dry matter content (method A). From this subset six pigmented animals (three En en + three En En, all with Cc heterozygosity) were used for electrophysiological and sodium transport studies. Additionally, measurements of the ashes of the faeces dry matter were performed and the blood was examined with respect to the hormones of the thyroid gland. Concentrations of T_3 and T_4 were analysed by a standardized

radioimmunoassay (Biocontrol, Mainz). All experiments with animals of the latter sample were performed at the same daytime (slaughtering of the rabbits at 9.00 a.m.).

The other subset of 14 animals of group B (six EnEn and eight Enen, $F_i = 0.125$) was integrated in this study for measurement of the dry matter content and the proportion of the ashes, using the whole intestinal wall (method B) for incubation (three segments: small intestine, cecum and colon).

For evaluation of electrophysiological parameters and transepithelial fluxes of sodium, the cecum was opened at the small curvature, the content was removed manually and the cecal wall was carefully rinsed with ice-cold buffer solution. The mucosa was separated from the underlying tissues by blunt dissection (FRIZZELL et al., 1976). The term 'epithelium' is used throughout this paper for the preparation obtained from this procedure. The epithelia were mounted between two halfs of Ussing-type chambers (3.14 cm^2 exposed area) under short-circuit conditions. Edge damage was minimized by silicon rings. The mucosal and the serosal side of the epithelia were incubated at 37 °C with the following buffer solution (in mM : Na^+ 140, Cl^- 124, HCO_3^- 21, K^+ 5.4, $H_2PO_4^-$ 2.4, Mg^{2+} 1.2, Ca^{2+} 1.2 and glucose 10. The solution was gased with a mixture of 95 % O_2 and 5 % CO_2.

The transepithelial potential difference (PD) was determined using two AgCl half-cells and a millivoltmeter connected with the mucosal and serosal side of the chambers by agar-gel bridges filled with buffer solution. The transepithelial conductance was determined by changes of the PD while currents of 100 µA, in alternating directions, were applied on the tissue by a computer-controlled device connected with the chambers via pairs of Ag-AgCl electrodes. The tissue conductance was corrected for the conductance of the buffer solution, measured before mounting the epithelia. The corrected conductances, together with the PD and the short-circuit current were printed out at regular intervals.

Experimental procedure

Ten min after mounting the epithelia, 2 µCi of ^{22}Na was given either to the mucosal or to the serosal buffer solution. The epithelia were allowed to equilibrate for 30 min. Samples of 100 µl from that side where the isotope was applied were withdrawn for determination of the specific activity of the isotope. At the beginning and at the end of each flux period samples of 500 µl from the unmarked buffer solution were taken for determination of the transepithelial unidirectional movements of sodium. Net fluxes of sodium were calculated as differences between the unidirectional fluxes of sodium in opposite directions ($J_{m\rightarrow s} - J_{s\rightarrow m}$) taken from epithelia with similar conductances. Measurements of two consecutive 30 min flux periods were taken to calculate average sodium fluxes.

Calculations

1. Unidirectional fluxes of sodium:

Flux of sodium (µmol) = $(cpm_{UE} \cdot V_U \div V_S + cpm_{UB} - cpm_{UE} \cdot V_U \div V_S) \div$ spec. activity

where cpm_{UE} = counts per min in the sample received from the unmarked incubation solution at the end of a flux period, V_U = fluid volume of unmarked incubation solution (ml), V_S = sample volume (µl) cpm_{UB} = counts per min in the first sample received from the unmarked incubation solution at the beginning of a flux period.

2. Specific activity of ^{22}Na:

$$(\text{counts } \mu\text{mol}^{-1}) = (cpm_M \cdot V_M \div V_S) \div M$$

where cpm_M = counts per min in the first sample taken from the ^{22}Na marked incubation solution, V_M = fluid volume of the marked solution (ml), V_S = sample volume (µl) and M = total amount of sodium in the marked incubation solution (µmol).

The data referring to morphological traits, pH-values and contents of dry matter resp. ashes have been analysed by Student's *t*-test. If the variance ratio test or Bartlett's test of Homogeneity of variance was indicating a different variability of the groups a modified *t*-test was used. The results are given by means ± SD. Additionally a Two Way ANOVA in a GLM procedure was performed to test for genotypical or methodical differences of the dry matter content of the bowels wall.

The results received from the electrophysiological studies and sodium flux experiments are expressed as means ± SEM. Differences between means were evaluated by Student's *t*-test or by the Mann-Whitney Rank Sum test. Differences were considered significant for $P < 0.05$.

Results

Table 1 presents some traits depicting different sizes of the body resp. organs

Table 1. Weight of the body, carcass, gut, adrenals and concentrations of T_3 and T_4 of En en and En En rabbits

	En en N = 14	En En N = 12	P
body weight (g)	4096 ± 399	3594 ± 287	**
carcass weight[1]			
absolute (g)	2044 ± 189	1651 ± 279	***
relative (%)	49.9 ± 1.71	45.6 ± 5.03	*t
gut weight			
absolute (g)	363 ± 37.4	563 ± 245	*t
relative (%)	9.02 ± 0.99	16.01 ± 8.16	**t
adrenals weight			
absolute (g)	0.231 ± 0.040	0.237 ± 0.066	NS[t]
relative (‰)	0.056 ± 0.009	0.067 ± 0.021	NS[t]
	N = 3	N = 3	
T_3 (ng/dl)[1]	148 ± 16	103 ± 22	*
T_4 (µg/dl)	5.13 ± 1.32	4.23 ± 1.18	NS

[t] Bartlett's test resp. the Variance Ratio test revealed departure from normal distribution; a modified *t*-test was used; N is the number of animals.
***, **, *, NS = $P < 0.001$, $P < 0.01$, $P < 0.05$, $P > 0.1$
[1] Sex differences on a significant level only have been found for the relative carcass weight. Whereas male rabbits had a portion of 46.4 ± 4.81 % of the body weight, females had a portion of 49.7 ± 2.47 % respectively, indicating that males were more afflicted than females but the T_3 values were lower in the latter.

Table 2. Transepithelial conductance and short-circuit current of cecal epithelia of En en and En En rabbits

	G_t $ms \cdot cm^{-2}$	I_{SC} $\mu eq \cdot h^{-1} \cdot cm^{-2}$	n
En en	6.09 ± 0.21	6.03 ± 0.20	36
En En	6.66 ± 0.40	4.34 ± 0.23*	24

Values are means ± SEM; n is the number of epithelia, * = $P < 0.0001$

dependent on the spotting type of slaughtered rabbits used for this series of investigations. Decreased body weights and carcass weights (absolute and relative) were genotypical features of En En rabbits. In contrast the weight of the gut was increased markedly especially when analysed in relation to the body weight at slaughter. A significant reduced value of Trijodothyronine in the blood of these animals was concomitant to these findings.

The short-circuit current (I_{SC}) and the transepithelial conductance (G_t) reached stable plateau values about 40 min after mounting. The average values measured during two consecutive flux periods are given in Table 2. Both groups of animals developed similar transepithelial conductances. The short-circuit current was significantly reduced in homozygous spotted animals by about 25 %.

Great differences of $J_{m \rightarrow s}$ and net fluxes of sodium were found between both groups of animals (Table 3). In ceci received from homozygous rabbits the sodium net flux was significantly reduced by about 50 %, but $J_{s \rightarrow m}$ fluxes was not significantly altered.

En En animals had a lower pH in the proximal duodenum. A markedly reduced

Table 3. Unidirectional fluxes of sodium and calculated net fluxes of sodium across the cecal epithelium of En en and En En rabbits

	Flux $J_{m\rightarrow s}$	$J_{s\rightarrow m}$ $\mu eq \cdot h^{-1} \cdot cm^{-2}$	J_{net}	n
En en	7.36 ± 0.39	2.46 ± 0.21	4.90 ± 0.28	17
En En	5.19 ± 0.48*	2.62 ± 0.32	2.56 ± 0.30*	9

Values are means ± SEM, n is the number of epithelia, * = $P < 0.05$

Table 4. pH values, dry matter of the faeces and ashes of the faeces dry matter in samples from ingesta of En en and En En rabbits

	En en N = 6	En En N = 6	p
	pH-value		
Duodenum	6.91 ± 0.09	6.63 ± 0.17	**
Jejunum	6.86 ± 0.19	6.93 ± 0.21	NS
Ileum	7.72 ± 0.18	7.52 ± 0.25	NS
Caput ceci	6.49 ± 0.44	6.52 ± 0.47	NS
Apex ceci	6.52 ± 0.43	6.39 ± 0.44	NS
	Dry matter of the faeces (%)		
Caput ceci	19.14 ± 2.81	16.15 ± 1.70	*
Apex ceci	19.73 ± 2.95	19.26 ± 0.90	NS[t]
Distal colon	25.71 ± 3.39	23.66 ± 3.06	NS
	Ashes of the faeces dry matter (%)		
	N = 3	N = 3	
Caput ceci	10.58 ± 0.26	12.25 ± 1.11	(*)
Apex ceci	10.20 ± 0.25	11.90 ± 1.11	NS[t]
Distal colon	9.65 ± 0.31	10.97 ± 0.93	(*)

Localizations: Duodenum: 4 cm behind the pylorus; Jejunum: 50 cm downstream the pylorus; Ileum.: 10 cm before the Sac. rotundus

[t]: Bartlett's test resp. the Variance Ratio test revealed departure from normal distribution; a modified *t*-test was used; N is the number of animals.

**, *, (*), NS = $P < 0.01$, $P < 0.05$, $P < 0.1$, $P > 0.1$

proportion of dry matter in the faeces was found in the ceci. In tendency En En rabbits had increased amounts of ashes within the faeces dry matter of the large intestine (Table 4). All traits of this set showed no sex differences.

The dry matter content of the intestinal wall measured by method A using small sheets from eight different locations was not significantly different between En en and En En rabbits (Table 5) and no sex differences were determined results not shown). It should be noted that the proportion of dry matter of the ileal wall was very low in both genotypes.

Comparison between the dry matter content of the intestinal wall (Table 6) showed no significant difference ($P > 0.05$) among both genotypes regardless which method for dry matter analysis was used. Thus running a Two Way ANOVA in a GLM procedure for comparison of the genotypes revealed P-values lower than 0.152 for all three segments of the bowel.

It should be noted that in En En rabbits the mean values of the dry matter content

Table 5. Dry matter content (%) of the intestinal wall. Values were obtained by method A using small biopsies from eight different locations at the gut

	En en N = 6	En En N = 6
Duodenum	18.08 ± 1.08	21.29 ± 7.39
Jejunum	20.27 ± 4.50	19.83 ± 5.70
Ileum	14.73 ± 2.39	16.12 ± 3.23
Caput ceci	17.15 ± 2.95	16.93 ± 4.33
Apex ceci	20.99 ± 5.48	16.06 ± 3.81
Colon, proximal		
3 folds	20.55 ± 5.15	17.83 ± 3.76
1 fold	21.16 ± 3.57	18.94 ± 3.56
Colon, dist.	19.29 ± 6.31	16.63 ± 2.46

N is the number of animals. Values of both genotypes did not differ significantly ($P > 0.1$).

Table 6. Dry matter and contents of the ashes of the bowel wall in different intestinal segments of En en and En En rabbits. Values were obtained by method A and method B

	En en N = 6	En En N = 6	En en N = 8	En En N = 6
	method A[1]		method B	
	Dry matter (%)			
small intestine	17.69 ± 1.66	19.08 ± 5.35	15.05 ± 2.85[2]	17.82 ± 2.14[2]
cecum	19.07 ± 3.10	16.50 ± 3.77	18.13 ± 3.71	14.65 ± 4.27
colon	20.33 ± 3.31	17.80 ± 2.60	17.81 ± 0.69	17.67 ± 2.12
			Ashes (%)	
small intestine			4.32 ± 0.78	4.59 ± 0.88
cecum			3.81 ± 0.86	3.83 ± 0.50
colon			3.97 ± 0.46	4.18 ± 0.88

[1] Values are calculated means ± SD based on measurements shown in Table 5.
[2] $P < 0.1$; N is the number of animals.

of the small intestine were about 13.2 % larger, but in the large intestine about 11.5 % smaller than the dry matter content of En en rabbits. Comparing these relative differences of means by Student's *t*-test elucidated a significant effect of the genotype on the dry matter content of the bowel between small and large intestine ($P = 0.0209$).

Though the mean values determined by method B in average were about 10 % lower than those determined by method A, these differences could not be considered to be significant when using the GLM procedure ($P > 0.1$). In addition, this procedure revealed no interaction between the different genotypes and the methods for evaluating the dry matter content.

Discussion

Previously the Megacolon-Syndrome of homozygous spotted rabbits (En En) has been characterized for its symptomatology due to genetical, morphometric and histomorphometric analyses. The trigger-point for the pathogenesis still has not been

found although some indications for endocrinologic and neurologic disturbances have emerged. Though this study was not performed to answer this question it could fill some further gaps in the understanding of the actions of the incompletely dominant 'English Spot' gene (En) or a closely linked recessive. All the genotypical features described in the introduction have had its relevance for this sample of investigated spot rabbits too. Table 1 emphasizes the undersize of En En rabbits at the age of 21 weeks.

The body weight at slaughter and the absolute weight of the carcass were beneath the values of En en rabbits on a significant level but the weight of the gut and in tendency the weight of the adrenals were markedly above the values of En en rabbits especially when examined in relation to the body weight at slaughter. A significantly reduced value of Trijodothyronine in the blood of slaughtered En En animals was concomitant with these findings. Due to the increased weight of the bowel, the offal of slaughterings were increased, too, and therefore the relative carcass weight is decreased in 'White Spot' rabbits. If a catabolic metabolism is brought about by this disease, lysis of muscle proteins and fat tissue causes unsatisfactory relative carcass weights but the relative weight of the gut increases overproportionally. This is a reason for a much broader standard deviation of many analysed traits in 'White Spotted' rabbits of this set also, and this interferes with some statistical tests. Thus the genotypical differences of the means of the adrenals relative weight are influenced by this and had to be interpreted as not significant. Considering the symptomatology of this disease, the analysed animals were regarded to be suited for studies on pathogenetical aspects of the Megacolon-Syndrome represented in this paper. Nevertheless, the cecum of one homozygous animal (not included in Tables 2 and 3) developed completely different transepithelial conductances (27.36 ± 9.52 mS cm^{-2}), but the short-circuit current was not significantly different from the values given in the table. In those epithelia extremely increased unidirectional fluxes were observed ($J_{m \to s}$: 16.2 ± 1.5; $J_{s \to m}$: 13.88 ± 2.18 μeq·h^{-1}·cm^{-2}) though the sodium net transport remained unaltered (2.46 ± 0.38 μeq·h^{-1}·cm^{-2}). This is a further hint for mediating a much broader standard deviation of some traits due to the defective gene in En En rabbits. Thus these effects should be interpreted as a genotypical feature characterizing the variability of disease proneness by further variance components reported elsewhere. The clinical phenomenology of this disease can be varied by the sex and the genetical background of the strains of bred rabbits and surely by the environment also. It is concluded that this variability of disease manifestation is of interest for the commercial rabbit meat production because albinism does not prevent this sickness, as shown by WIEBERNEIT and WEGNER (1995). The symptomatology of the Megacolon-Syndrome thus is comparable though not identical with some other disorders of the rabbit's bowel with still unknown aethiopathogenesis (GÖRSE, 1994) such as 'Intestinal Paresis' of breeding does or 'Mucoid Enteropathy' of broiler rabbits (LEBAS et al., 1984; OKERMAN, 1988). This is of relevance since albino rabbits are often used for rabbit meat production (on a WNS or Californian background). Nevertheless, it is imaginable that the 'Megacolon-Disease' could occur in totally pigmented rabbits too, but this would be possible only if a defective gene closely linked to En in spot breeds would recombine to en in self-coloured rabbits due to crossing over. Till now the local stock of spotted rabbits (about 1000 rabbits were bred from six generations of breeding does) rendered no corroboration of this hypothesis; in addition the literature gives no specific hints for the incidence of this bowel disease in rabbits with a completely pigmented fur.

Obstipation of the cecum can be the final feature of this disease. This occurred in purebred breeding does after weaning from their litters and sporadically in a few broiler rabbits during the fattening period. The initial site of obstipation was the apex of the cecum whereas complication from diarrhea was seen in one case only. Since the proximal colon was empty in homozygous DRS does, which requires peristaltic and anterograde movements of the colon wall for the transport of the ingesta, and the obstipation (but not the poor development) of two severely diseased broiler rabbits

responded to therapy by application of paraffin oil and massages of the abdomen several times a day, it is concluded that hypoganglionosis of the distal colon is unlikely to be the cause of this kind of megacolon of rabbits but could be a symptomatic feature. This view is supported by the fact that the intestinal wall at a proximal duodenal location was hypoganglionotic for the Pl. myentericus (WESSEL, 1993). Likewise as in man's 'Hirschsprung Disease' (a group of diseases caused by a lack of ganglia in the myenteric and submucosal plexus, in its classical form the 'Aganglionotic Colon') (KAISER and BETTEX, 1982) it should be mentioned that the 'hypoganglionotically' caused dilatation of the rabbits bowel should be cranial of such a location. Thus neuroendocrine interactions may exist. This is of interest for comparative studies and for differential diagnosis of diseases with a megacolon as a cardinal symptom in other species of 'spotted' animals such as horses (MCCABE et al., 1990) and mice (LANE and LIU, 1984) or in man for whom already many 'Hirschsprung' like symptomatologies have been described (SCHÄRLI, 1982). Recent investigations also found no direct participation of the central nervous system in the pathogenesis of megacolon in spotted rabbits (KÜHNEL, 1994; REICH, 1995).

The cecum of the rabbit has been described as a tissue in the range between a leaky and a moderately tight epithelium. Transepithelial conductances between 6.4 and 11.1 $mS \cdot cm^{-2}$ were measured, and the short-circuit current varied between 3.28 and 9.2 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ (HATCH, 1987; HATCH and FREEL, 1988; CLAUSS et al., 1985; TAI et al., 1989; CLAUSS et al., 1989). The rabbit cecal epithelium is characterized by a large absorption of sodium. The cited authors measured net absorption rates for sodium ranging from 3.3 to 9.0 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$. The transcellular sodium transport and the short-circuit current is mainly due to an electrogenic phenamil sensitive transport (SELLIN et al., 1988) depending on the time of day (CLAUSS, 1993). The electrophysiological parameters and the transepithelial net fluxes of sodium for heterozygous spotted rabbits presented here (Tables 2 and 3) are in good agreement with the data previously reported by those authors. Unidirectional fluxes of sodium determined in the experiments were somewhat lower than reported by most authors. As has been mentioned by HATCH and FREEL (1988) due to several differences with respect to the results from CLAUSS (1985), the period of faeces production may cause such variability between different investigations. In contrast to the cited authors' experiments, which were performed 4 h after onset of the lightening period using epithelia from anaesthetized animals (pentobarbital), we used epithelia from rabbits slaughtered 3 h after onset of light. All preparations were done within maximal 10 min after stunning. Although the experiments were performed exactly at the same time after onset of the light, two animals (En en) were found to be in the hard faeces period and one animal (En en) had soft faeces in the distal colon, but one En En rabbit exhibited hard, one soft faeces and one was in the transition phase. Therefore we conclude that the structure of the faeces is not exclusively synchronized with the lightening period. This may be a further reason for different absorption rates of sodium in our experiments.

In homozygous spotted rabbits the net transport of sodium was reduced by about 50 % due to a significantly diminished J_{ms} underlined by simultaneously reduced short-circuit currents. In most homozygous spotted animals used in this study, a dilatated cecum has been observed. Therefore the reduced net transport rates of sodium and the reduced short-circuit currents could have been due to a stretched and therefore thinner cecal epithelium. In this case the transepithelial conductance and, consequently, the passive part of the sodium transport (mainly reflected by the unidirectional flux rates of sodium from the serosal to the mucosal side) should have been increased. But the epithelia which were received from En En animals showed neither significantly altered transepithelial conductances nor enhanced s→m transport rates compared with epithelia received from heterozygous animals.

As in many other epithelia in the rabbits cecum active sodium transport is driven by a basolateral located ATP-dependent sodium pump which extrudes sodium from

the intracellular compartment resulting in an inward directed electrochemical gradient for sodium at the apical membrane (CLAUSS et al., 1989). Sodium enters the cells mainly electrogenically through a phenamil sensitive pathway (SELLIN et al., 1988), whereas the electroneutral entry process of sodium via a Na^+/H^--exchange amounts only for about 10 % of the total sodium transport (CLAUSS, 1993). In our experiments the reduction of net sodium transport observed in En En rabbits was about 50 %. Thus it is concluded that the electrogenic component of transcellular sodium transport must be affected in homozygous spotted animals. This view is supported by the fact that the I_{SC} was reduced simultaneously.

This study was performed with animals of a defined genetic background and the local conditions for keeping and housing rabbits offered standardization and welfare to them. Considering this vital heterozygous animals offered a good reference for comparisons with the defective homozygous animals as well as with the data obtained from literature. Hence we believe that the results presented here have relevance for the discussion of pathogenetic features of the Megacolon-Syndrome.

As an effect of the reduced sodium absorption from the ingesta, its content of dry matter was significantly reduced at the caput of the cecum by about 3 % whereas comparable portions of dry matter were found within the ingesta of the apex ceci (Table 4). It is of interest that the appendix ceci (Proc. vermiformis) secretes a liquid rich in bicarbonate into the apex ceci and that the profusion resp. obstipation of the cecum starts within the apex. Furthermore, appendices of affected animals were lower in weight. This could have been caused by a reduced contact with cecal contents containing bacteria necessary for the proliferation of its epithelia as it was concluded by BLYTHMAN and WAKSMAN (1973) after experimental ligation of the appendix ceci.

Another effect of the altered ion absorption may be represented by increased (in tendency) ash contents of the faeces of the large intestine by about 2 % in comparison with heterozygous spotted animals; but the pH values in different locations of the bowel are unchanged, with one exception: at the beginning of the duodenum the pH value of the ingesta is significantly decreased in homozygous spotted rabbits. Probably the ingesta leaving the stomach is more acidic in En En rabbits. On the other hand, this could be a reason for reduced infection rates with the worm Passalurus ambiguus haunting the cecum of rabbits as reported by GROSSE HACKMANN (1994).

One characteristic symptom of this disease apparently consisted in a significantly more liquified ingesta in the cecum and in the distal colon ($P < 0.1$) in disposed animals but severely affected animals suffered from obstipation. It is concluded that a hormonal imbalance changes the sodium transport capacity in En En genotypes. This would be underlined by the hypothyreotic condition and by hypertrophic adrenals in these animals and is supported by the results given in the Tables 5 and 6. Whereas the proportion of dry matter content of the wall of the small intestine was increased in 'White Spot' rabbits, its proportion was decreased within the large intestine (esp. the cecum). The latter could be interpreted by a myxedematous swelling of the gut wall due to reduced values of Trijodothyronine in the blood; but the cause for the increase of dry matter in the small intestine still remains unclear. In this context it seems to be of interest that the length of the small intestine was significantly reduced in En En crossbred rabbits (second crosses of ES and DRS) by 30 cm (GROSSE HACKMANN, 1994). In this generation the body weight at slaughter and the carcass weight (absolute and relative) of En En rabbits were nearly identical with En en ones (GÖRSE, 1994), thus indicating enhanced vitality due to heterosis. In addition the absolute and relative weights of the wall of the small intestine showed no remarkable alteration (but the large intestine expressed all signs of the predisposition of the Megacolon-Syndrome, i.e. increased absolute and relative weights of the gut wall). This implies that the specific weight and the strength of the wall of the small intestine is increased in relation to its length as it could be deduced for the small intestine (especially for proximal compartments) by the results of an investigation performed by WESSEL (1993). From this point of view it is imaginable that the shortened but thickened small intestine is

just as meaning for shifting this syndrome from predisposition to disease as is the hypertrophy of the adrenal cortex and otherwise a hypothyreotic state of metabolism. Since the shorter small intestine already occurs in well developed En En rabbits this organ could be another potential site of an early gene action (either directly or pleiotropic), defecting developmental growth of the small intestine. By altered conditions for the absorption of nutrients together with a catabolic state of metabolism severe 'downstream' effects would be explicable.

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