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## CHANGES IN HYDROMINERAL METABOLISM IN DIARRHOEIC RABBITS

### 1. A study of the changes in water metabolism.

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#### Résumé

LES MODIFICATIONS DU METABOLISME HYDROMINERAL CHEZ LE LAPIN ATTEINT DE DIARRHÉE. 1. *Etude du métabolisme hydrique.* — Les diarrhées du lapereau provoquées par infestation expérimentale avec des coccidies se manifestent par l'excrétion réduite de fèces dont la teneur en eau n'est que faiblement augmentée. Les consommations d'aliment et, dans une moindre mesure, d'eau, diminuent. Il en résulte une hydratation du bol alimentaire qui se traduit par une teneur en eau élevée dans l'estomac et le caecum. Par contre, l'eau est très bien réabsorbée au niveau du colon distal. La diurèse est peu modifiée, l'hématocrite s'abaisse indiquant une tendance à l'hémodilution. Il n'y pas de déshydratation globale des animaux ni de modification du secteur hydrique extracellulaire. La teneur en eau totale du muscle s'élève légèrement tandis que la peau se déshydrate de façon importante.

#### Introduction.

Digestive ailments are common in young rabbits during the growing period and the resultant morbidity and mortality are a source of great concern in rabbit breeding (Löfliger *et al.*, 1969; Whitney, 1970; Vetesi and Kutas, 1973; Renault, 1975).

Apart from intestinal coccidiosis, it is difficult to define the different forms of enteritis etiologically responsible (Mack, 1962; Whitney, 1976) and, whatever the agents responsible, the clinical manifestations are often similar (Coudert, 1975). Rollins and Casady (1967) reported that 75% of digestive ailments take the forme of diarrhoea.

In view of these difficulties, we decided to tackle the problem from the physiopathogenic aspect, and examine any disturbances of hydromineral metabolism which might occur during diarrhoea.

One of the hypotheses which the work is based on is that the physiopathogenic observations during diarrhoea are always the same whatever their etiology. We therefore decided to start with a study of coccidia-induced diarrhoea. Some coccidia have the advantage of consistently inducing diarrhoea (Coudert, 1975), whereas Hoffman *et al.* (1973) showed that *Escherichia coli* alone did not induce reproducible diarrhoea in the rabbit.

In most species, this diarrhoea generally leads to dehydration which is characterized by the persistence of a skin fold and by metabolic acidosis before death (Fayet, 1968 b and c; Bernheim *et al.*, 1964). There is little information concerning changes in the hydromineral metabolism in the rabbit. Some authors have drawn attention to biological disturbances, in particular to the changes in plasma composition (Kruiningen and Williams, 1972; Kutas *et al.*, 1973). In order to define quantitatively the diarrhoeic state and to study the extent of the disturbances of the hydromineral metabolism, water and the main electrolytes have been determined in the feed, feces, urine, blood plasma, and in various parts of the digestive tract content. Using the same rabbits, we measured with dilution techniques the distribution of water in the whole animal, and in some specific tissues (muscle and skin).

This first article concerns only the results for the water.

## Materials and Methods.

### 1. ANIMALS AND ANIMAL HOUSES

All rabbits used were from the Fauve de Bourgogne strain and came from a protected breeding station where there had been no past pathological record for several years. They were aged between 7 and 9 weeks. This is a period just after weaning during which diarrhoea is most prevalent and most serious.

The experiments were carried out in a protected environment (constant temperature of 16°C, dynamic ventilation, air filtered at 10 $\mu$  and maintained at a pressure slightly higher than atmospheric). The rabbit house was disinfected after each experiment with steam under pressure at 120°C. The cages were made entirely of metal grating and were thus easy to disinfect. Each cage had an automatic drinking bottle. Food was distributed *ad libitum* in the form of whole granules, free from antibiotics and anticoccidian agents.

### 2. EXPERIMENTAL INDUCTION OF DIARRHOEA

Diarrhoea was experimentally produced by orally infecting the rabbits with coccidia; two different species were used—*E. intesti-*

*nalis* (Cheissin, 1948) and *E. pellerdyi* (Coudert, 1977). Between 30 and 80,000 oocysts, depending on the species, were introduced directly into the stomach, through a tube.

### 3. CLINICAL MANIFESTATIONS

#### *Diarrhoea.*

The development of the diarrhoea was empirically assessed; the apparent fluid content of the feces was quoted daily from 0 to 2.

#### *Loss of cutaneous elasticity.*

The persistence of a skin fold after pinching 3 to 4 cm of the skin in the lumbar region was quoted from 0 to 3.

#### *Weight gain.*

The animals were weighted three times per week.

### 4. MEASUREMENT AND DETERMINATION OF INGESTA AND EXCRETA

The food and water intake was measured three times per week. This enabled us to assess the total amount of water ingested (drinking water plus water contained in food). The faeces and urine were measured daily. The faeces were dried in a crucible at 90°C for 48 hrs (the duration and temperature of drying were fixed in preliminary tests, according to the characteristics of this material). To avoid the effects of natural drying of faeces which takes place during the night, the water content of faeces collected from the rectum was measured and the water excretion calculated from the total dry matter excreted during the day.

### 5. EVALUATION OF THE WATER CONTENT IN DIFFERENT PARTS OF THE DIGESTIVE TRACT

The animals were slaughtered by rupturing the cervical vertebrae. The abdomen was opened and the digestive tract was clamped with Kocher forceps at various points so as to separate the 7 segments corresponding to the following anatomical divisions: stomach, duodenum, jejunum, ileum, caecum, proximal colon and distal colon. This oper-

ation was carried out rapidly so as to avoid any post mortem transfer of intestinal content between the different segments. The contents were removed by gently squeezing each segment of the intestine between the fingers, and were collected in a tube. They were then weighed and centrifuged. The plug was dehydrated in an oven at 90°C for 24 hours.

## 6. METHODS USED TO INVESTIGATE THE LIQUID COMPARTMENTS

All measurements were made on the 10th day after inoculation when the syndrome was most acute.

### a) *Volumes of total body water and extracellular water:*

—total water.

In the rabbit, there is a large amount of intestinal contents because of the great capacity of the digestive reservoirs such as the stomach and the caecum.

We removed the intestine in order to avoid including the gut water content in total body water.

After the intestine was removed, the whole animal was frozen at -40°C, pulverized and then homogenized. Total water was calculated by drying a sample of 150 g at 80°C for 48 hrs.

—extracellular water.

The method used involved diffusion of a

sulphate ion labelled with sulphur 35 (Tournadre, 1973).

In the preliminary experiments on one group of healthy rabbits and on one of inoculated animals, it was determined from the method of extrapolation of Cachera and Barbier (1941) that the expansion volume of  $^{35}\text{SO}_4$  was reached 11 minutes after injecting sulphate in the animals of both groups. It was checked that the urinary and intestinal excretion was then negligible.

The animals were first of all weighed and then the sulphate solution ( $\text{Na}^+_2(^{35}\text{SO}_4^{--})$  ISOTEC) was injected intravenously with 10  $\mu\text{Ci}$  per kg of live weight. An intracardiac blood sample was taken 11 minutes later and this was centrifuged. An aliquot of plasma was dehydrated at 85°C for 24 hrs so as to determine the plasma water content. The radioactivity of 100  $\mu\text{l}$  of plasma was measured by liquid scintillation (Mark, Nuclear Chicago). The extracellular volume was calculated by use of the following equation:

$$V = 1.111 t Q / C$$

where  $V$  = diffusion space in ml;

$Q$  = amount of  $^{35}\text{SO}_4$  injected in dpm;

$C$  = plasma concentration of  $^{35}\text{SO}_4$  in dpm/ml;

$t$  = plasma water content;

1.111 = Donnan factor for the  $\text{SO}_4$  ion (Van Leeuwen, 1964).

### b) *Haematocrit.*

The haematocrit test was carried out on the blood samples used in the previous test.

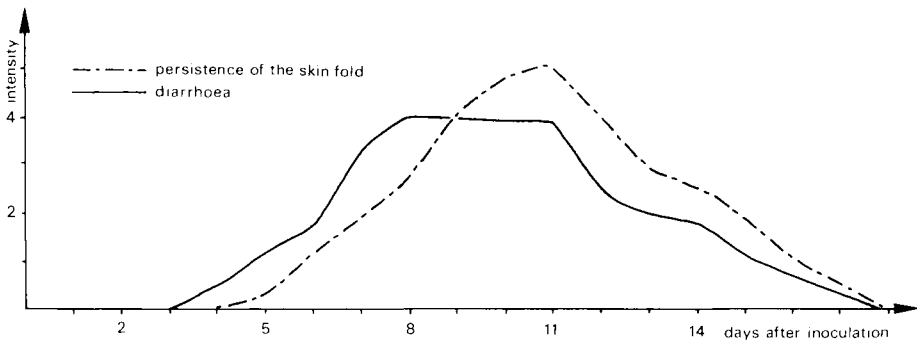


Fig. 1—Evolution of the symptoms of diarrhoea, and the persistence of the skin fold (arbitrary quotation).

c) *Total muscle and skin water.*

Two muscle samples and one skin sample were taken from all animals used in the previous tests, which were killed 10 days after inoculation. Each sample was weight and then dehydrated at 60°C for 16 hrs, under vacuum in order to burst the cells. Measurement of the dry weight enabled us to calculate the total water content.

7. STATISTICAL ANALYSES

All statistical tests carried out were variance analyses except for those values calculated from a ratio (percentage) where a covariance analysis was chosen in order to take into account the difference in the size of the samples (Snedecor and Cochran, 1971).

Results.

1. DEVELOPMENT OF THE DIARRHOEA AND OF THE SKIN TEST (fig. 1).

Although difficult to measure, these two symptoms are useful because of their reproducibility and the time of their appearance.

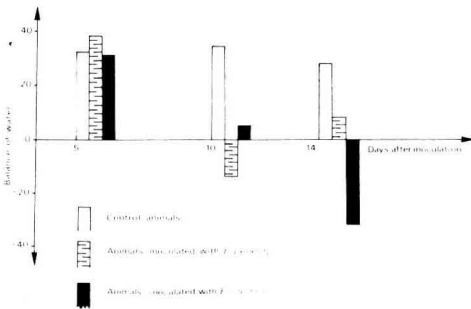


Fig. 2—Water balance (water ingested less fecal and urinary water) in young rabbits inoculated with *Eimeria pellerdyi* and *E. intestinalis*, 5, 10 and 14 days after inoculation.

The “dynamics of diarrhoea” can be resumed thus: a period of establishment characterized by a progressive loosening of the faeces, a relatively short acute phase and then a period of slow recovery.

It can be remarked that the evolution of the persistence of the cutaneous wrinkle was much the same as that of diarrhoea except that it was one day behind. The maximum was reached at the end of the acute phase (day 11).

2. WATER BALANCE

a) *Food and water consumption* (table 1).

In the control group, the average consumption of drinking water was 159 g/day and of food 108 g/day. In animals suffering from diarrhoea (day 10), the total amount of

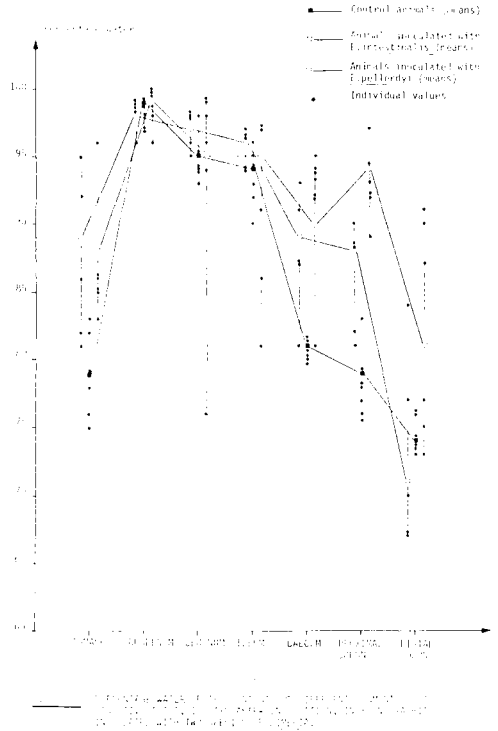


Fig. 3—Percentage water of the content of various segments of the digestive tract, 10 days after inoculation, in young rabbits inoculated with two species of *Eimeria*.

Table 1.—Evolution of weight gain and amounts of water and dry matter ingested in young rabbits inoculated with *E. intestinalis* and *E. pellerdyi*, 5, 10 and 14 days after inoculation ( $m \pm sm$ ).

DAYS AFTER INOCULATION	D3 to D5			D8 to D10			D12 to D14		
	INOCULATED ANIMALS			INOCULATED ANIMALS			INOCULATED ANIMALS		
	CONTROLS (7)	<i>Eimeria pellerdyi</i> (8)	<i>Eimeria intestinalis</i> (8)	CONTROLS (7)	<i>Eimeria pellerdyi</i> (8)	<i>Eimeria intestinalis</i> (6)	CONTROLS (7)	<i>Eimeria pellerdyi</i> (6)	<i>Eimeria intestinalis</i> (5)
Number of measurements									
Weight gain (g/24 h)	37,3 ± 3,6	29,6 ± 3,1	38,0 ± 3,9	32,3 ± 2,8	-4,10 ± 16,1	-52,6 ± 8,9	34,4 ± 8,2	10,6 ± 10,2	-0,4 ± 22,5
Food dry matter (g/24 h)	102,4 ± 5,3	67,2 ± 7,4	88,6 ± 5,6	101,4 ± 3,2	33,9 ± 10	13,7 ± 6,7	105,1 ± 7,8	52,5 ± 15,8	40,8 ± 24,6
Water contained in food (ml/24 h)	5,4	3,5	4,6	5,3	1,8	0,7	5,5	2,7	2,1
Drinking water (ml/24 h)	158,1 ± 10	139,3 ± 3,6	140,7 ± 4,5	173,2 ± 17,2	113,5 ± 18,5	72,7 ± 15,2	146,2 ± 14,2	125 ± 26,0	64 ± 32,4
Water ingested/dry matter ingested (ml/g)	1,59	2,12	1,64	1,76	3,40	5,33	1,44	2,43	1,62

Table 2.—Evolution of the amounts of urine and faeces excreted by young rabbits, 5, 10 and 14 days after inoculation (m±sm).

DAYS AFTER INOCULATION	5		10		14	
	CONTROLS	INOCULATED ANIMALS <sup>(a)</sup>	CONTROLS	INOCULATED ANIMALS <sup>(a)</sup>	CONTROLS	INOCULATED ANIMALS <sup>(a)</sup>
Urine (ml/24 h) (Number of measurements)	30.8±7.9 (8)	32.8±5.5 (16)	63.2±18.1 (8)	57±7.7 (14)	49.1±9.5 (8)	40.9±9.1 (11)
Water of rectal contents (g water/g dry matter)	2.48±0.33 (6)	2.57±0.11 (12)	2.16±0.10 (6)	3.86±0.73 (10)	2.47±0.06 (6)	3.55±0.42 (9)
Theoretical faecal water <sup>(b)</sup> (ml/24 h)	100.4	81.8	76.9	49.9	96.7	68.8
Faecal dry matter (g/24 h) (Number of measurements)	40.4±3.4 (8)	31.8±2.6 (16)	35.5±3.6 (8)	12.9±2.6 (15)	39.1±3.5 (8)	19.4±5.0 (8)
Faecal water/Faecal dry matter (ml/g DM)	2.48	2.56	2.16	3.85	2.47	3.55

(a) The values indicated represent the averages of the results obtained with *E. intestinalis* and *E. pellerdyi*.

(b) The theoretical faecal water was calculated from the excreted faecal dry matter and the water of rectal contents.

water ingested during the most severe stage diminished by 35% compared with the control animals for the group inoculated with *E. pellerdyi*, and by 59% for the group inoculated with *E. intestinalis*. At the same time, the consumption of dry matter decreased but to a proportionally greater extent than that of water so that there was a greater hydration of the food bolus.

#### b) Faecal and urinary excretion (table 2).

Urinary excretion altered very little during diarrhoea. The quantity of faeces diminished and, despite the fact that on average they contained 10% more fluid, water loss through diarrhoea was less than in healthy animals. Figure 2 shows that, although there was a reduction in the amount of water lost, there was a deficit in the water balance in sick animals. Control animals always had a positive water balance, sometimes this balance became negative in young rabbits inoculated with certain species of coccidia.

### 3. WATER IN THE GUT CONTENT AT DIFFERENT LEVELS OF THE DIGESTIVE TRACT (fig. 3).

The evolution of the water content of the food bolus along the intestine in the healthy animals was similar to that reported by Alexander (1965), Catala (1976) and Licois and Coudert (1976).

Only in the stomach, caecum and proximal colon was the water content much higher in animals suffering from diarrhoea, 10 days after inoculation. The hydration of the stomach content, which was practically doubled in sick animals, was solely a result of reduced food consumption. The water content of the intestine from the duodenum to the ileum did not seem to be altered.

On the other hand, whatever type of *Eimeria* was inoculated, water reabsorption in the caecum and the proximal colon was always reduced. This had the effect of producing watery faeces despite a compensatory reabsorption in the distal colon. However, it should be noted that individual variations are often very large and are greater in young rabbits suffering from diarrhoea than in the controls.

Table 3.—Water partition in the whole body, water content in some tissues and haematocrit of young rabbits inoculated with *E. intestinalis* and *E. pellerdyi*, 10 days after inoculation ( $m \pm sm$ ).

TREATMENTS	WHOLE ANIMAL				PLASMA Water %	MUSCLE Total water % (a)	SKIN Total water %
	Total water %	Extracellular water % (a)	Haematocrit	Water %			
CONTROLS (Number of measurements)	63.7 ± 0.8 (7)	20.6 ± 0.4 (28)	41.7 ± 0.3 (7)	93.1 ± 0.1 (7)	76.1 ± 0.1 (48)	70.5 ± 0.4 (7)	
INOCULATED ANIMALS (Number of measurements)	64.6 ± 0.6 (12)	21.1 ± 0.5 (33)	35.1 ± 0.6 (12)	90.7 ± 0.5 (12)	77.3 ± 0.1 (58)	63.6 ± 0.7 (12)	
STATISTICAL SIGNIFICANCE	0.01 < P < 0.025 (b)	N S (b)	P < 0.001 (d)	P < 0.005 (b)	(b) (c)	0.005 < P < 0.01 (b)	

(a) The values indicated represent the averages of the results of 3 identical trials.

(b) Covariance analysis taking into account the variations in sample size.

(c) A significant difference between groups was found in only one trial.

(d) Variance analysis.

#### 4. THE LIQUID COMPARTMENTS (table 3).

##### a) Total water and extracellular water volumes.

In the control group, the total water volume corresponded, on average, to 63.5% of the live body weight. The values were, of course, lower than those presented by Rico *et al.* (1967), as intestinal contents were excluded from our measurements. They were comparable to those obtained by Mehran and Gagnon (1966) in rabbits weighing from 2 to 3 kg. In animals suffering from diarrhoea, this volume was slightly higher (64.1%), but since the individual variations were great, the difference was only slightly significant ( $P < 0.025$ ).

As regards the extracellular compartment, the results obtained with the sulphate ion, which would seem to be the best adapted for the rabbit, were similar to those of Tournadre (1973). The values indicated in table 3 were calculated from several experiments. Although, on an average for each experiment, the volume of extracellular water was always higher in sick animals than in controls, the data was so scattered that it was impossible to show a difference between the two groups.

##### b) Haematocrit and plasma water.

The haematocrit fell by 16% ( $P < 0.001$ ). In the rat, the mouse and the dog, coccidia-induced diarrhoea is always accompanied by a significant increase of the haematocrit during the acute phase of the sickness (Heil, 1974). The plasma water content was very significantly lower ( $P < 0.005$ ) in inoculated animals. In fact, figure 4 shows that the plasma dry matter per unit of whole blood increased by 50% in animals suffering from diarrhoea. The plasma water rose by 8.5%, indicating a haemodilution.

##### c) Total muscle and skin water.

The total muscle water content was slightly higher in sick rabbits than in healthy ones. A covariance analysis in two tests did not reveal whether this difference was significant. However, in a third test the difference was found to be highly significant.

Compared to the healthy animals, there was a large drop of 10% in skin water content of the sick animals ( $P < 0.01$ ).



An attempt was made to evaluate the extra cellular volume of the muscle and skin but numerous problems of methodology (standardization of the samples) and technique (absorption of radioactivity by the muscle or skin extract) were encountered, which nullified the results.

### Discussion.

It is possible to induce diarrhoea with *E. pellerdyi* and *E. intestinalis*; the first species settles in the epithelial cells of the ileum (Cheissin, 1958), whereas *E. Pellerdyi* colonises the crypts of Lieberkühn of the caecum and the colon (Coudert, 1977).

Despite the fact that these two areas of the intestine have fundamentally different metabolic and physiological roles, similar results were obtained in all of the criteria studied. It is therefore possible that the young rabbits' diarrhoea is caused by non-specific, physiological or metabolic disturbances resulting from the aggression rather than by a definite etiological entity.

The diarrhoea was characterized first of all by a loosening of the faeces. These were less abundant between days 8 and 12. The result was a small water loss through the faeces, which is contradictory to the definition of diarrhoea, i.e. more frequent and

more liquid emissions accompanied by a mineral loss (according to Cottereau and Laval, 1975, in the calf).

It should be noted that, in establishing the water balance, the water lost by respiration or by perspiration was not taken into consideration.

The fact that diuresis altered little during diarrhoea suggests that there was no attempt by the animal to retain water. This, together with the lowering of the haematocrit and proteinaemia (Coudert *et al.*), indicated a haemodilution.

The hypoproteinaemia contradicted the reduction in of the percentage of plasma water measured, but the latter was explained by a considerable hyperlipaemia (Coudert *et al.*). It is very probable that the 50% increase in plasma dry matter was, among other things, linked to these lipids.

The disproportion between the water and dry matter ingested may partly explain the great hydration of the contents of the stomach and caecum. On the other hand, the return to sub-normal values in the distal colon suggested an efficient reabsorption in this area. It would now be interesting to determine what proportion of the water in the various parts of the digestive tract is endogenous in origin so as to be able to determine that provided by the food.

The total percentage of water in the organism was not affected by the diarrhoea; the animals' loss of weight, which was 10% after 3 days in some cases, was thus due to a proportional loss of water and of organic and inorganic substances.

In the animal as a whole, the distribution of water between the compartments was not altered by the diarrhoea either, as the volumes of extracellular water were much the same in control animals as in the sick ones.

On the more localized level of muscle and skin, it was seen that there was a definite slight increase in the total muscle water in contrast to an extensive dehydration of the skin. It is probable that the skin, which represents 14% of the rabbit's live weight, has the function of regulating an easily displaced water store. The persistence of the cutaneous wrinkle in this species is not a sign of extracellular dehydration, as is generally accepted by pathologists, but was due to the localized dehydration of this organ. It would be interesting to know whether or not this cutaneous

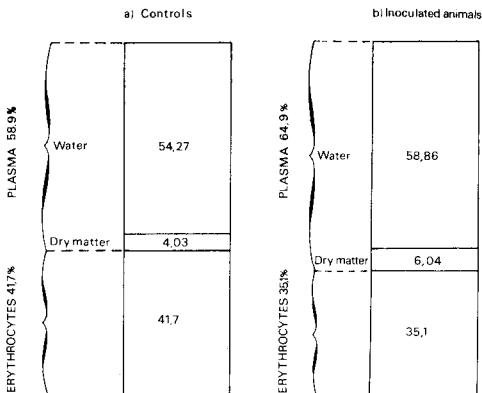


Fig. 4—Variations in haematocrit, plasma water and plasma dry matter (percentage) in animals inoculated with *E. intestinalis* and *E. pellerdyi*, 10 days after inoculation.

dehydration is significant in the pathogenesis of diarrhoea.

In comparing different factors of water metabolism in healthy rabbits and those suffering from diarrhoea, it was found that there were only a few features in common with diarrhoea in other species such as the calf or the infant. Studies involving these two species have revealed that the pathogenesis of diarrhoea is essentially tied up with hydromineral disturbances triggered off by digestive problems (Lafourcade *et al.*, 1964; Massip, 1976). In the case of the calf or the infant, diarrhoea was always characterized by a large increase in losses of faecal matter and, above all, faecal water (Fayet, 1968a; Lewis and Phillips, 1972; Fisher and de la Fuente, 1972; Lafourcade *et al.*, 1964). Diuresis was generally reduced, if not altogether absent (Fayet, 1968a; Gorin *et al.*, 1964) and there was a haemoconcentration (Phillips *et al.*, 1971). As regards water metabolism, the pathogenesis of diarrhoea was explained in a general way by extracellular dehydration (Fayet, 1968c; Bernheim *et al.*, 1964).

It has been seen that in the rabbit faecal and water excretion was less in sick animals than in healthy ones. Reabsorption was excellent in the distal colon. Diuresis was normal and there was no haemoconcentra-

tion. There was neither a general dehydration nor a change of the extracellular compartment. The only differences, as regards water, between healthy animals and those inoculated, concerned the *ingesta*, which were reduced, and the extensive cutaneous dehydration.

It is thus evident that the explanations of the physiopathogenesis of diarrhoea in the calf or infant do not apply to the rabbit. It should be noted that there was *a priori* an important difference related to age: diarrhoea in the calf and the infant affected the newborn whose food was entirely liquid, whereas the enteritis in the rabbit arose after weaning, in animals whose food was exclusively solid.

To aid an accurate interpretation of these results, the analysis of the water balance was completed by the study of any electrolytic modifications (Licois *et al.*).

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#### Summary

Diarrhoea was induced in the young rabbit by experimental infection with coccidia. It was characterized by a reduced excretion of faeces whose water content was only slightly increased. There was a drop in the consumption of food and, to a lesser extent, of water. There was a resultant hydration of the food bolus which led to a raised water content in the stomach and caecum.

Conversely, water was very efficiently reabsorbed in the distal colon. Diuresis was hardly affected at all. Haematocrit values were lower, which indicates a tendency towards haemodilution. The animals underwent neither general dehydration nor modification of their extracellular water. The total water content of the muscle rose slightly, whereas the skin was dehydrated to a large extent.

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