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RADIOACTIVE POLYETHYLENEGLYCOL : AN INTESTINAL WATER MARKER IN BIRDS

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SUMMARY

Polyethyleneglycol (MW = 4 000) labeled with ^{14}C remains in the liquid phase whatever the physical conditions are : dilution, alkalinity or acidity. It does not enter the food particles and is not adsorbed by them.

When the animals are in steady state conditions, a four-day balance study in four laying hens shows that ingested radioactivity is totally recovered in the feces. PEG-^{14}C is a valuable water marker in the bird intestine.

INTRODUCTION

Different markers have been tried in birds to measure intestinal transit as well as nutrient movements. Chromium oxide is considered as a solid phase marker and radioactive yttrium follows about the same particle size. None of them can be used for water and dissolved ion movement.

The first property required for a marker is to follow the element to be studied along the gut without being absorbed : a water marker has to be in the liquid phase, whatever the physical or chemical conditions of the solution are, and if possible, in a steady state situation.

In this work several *in vitro* assays have been done to test the solubility of ^{14}C-PEG (1) in different physical conditions, and to measure PEG balance *in vivo.*

(1) Polyethyleneglycol uniformly labeled. MW : 4000.

RESULTS AND DISCUSSION

1. — *Effects of water dilution and pH*

Due to water movements, food dilution varies widely along the digestive tract ; the ratio between water and dry matter ranges from about 2 in the ingesta to 8 in the duodenum content. Furthermore, the titratable acidity of secretions changes pH conditions : in crop and gizzard the pH goes lower than 3 and may increase to 8 in the distal part of the ileum.

In a factorial model, the effects of these two parameters on calculated water volume have been tested *in vitro* on 5 g of food dried at 110°C (table 1).

TABLE I

In vitro *Effects of water dilution and pH on the calculated water space occupied by PEG-^{14}C*

pH	Volume in ml of radioactive water mixed with 5 g of food		
	10	25	40
< 3	9.70 (1)	24.37	40.03
	9.55	24.16	39.46
> 8	10.05	24.83	39.60
	9.77	24.78	39.34

(1) In the two trials each space was calculated from 4 different counting vials.

Neither dilution nor pH seemed to have effect ; PEG remained in the liquid phase no matter what the pH was.

However, most of the calculated space was lower than the theoretical added volume.

2. — *Effect of food hydration*

In the foregoing trial, water content was minimized by predrying the food. Another assay was done on 2 g of food hydrated first with cold water or dehydrated (table 2).

With the control food (12.4 p. 100 water) and the prehydrated food (two parts water for one part food), PEG space was only 0.7 p. 100 lower than the added volume. As these two samples represent the normal process in the ingestion and hydration of food in the digestive tract, we may conclude that PEG space error is almost null.

However, with dehydrated food, the estimated space was 4 p. 100 lower. This indicates that water, hydrating the food, enters without radio PEG.

From these two trials we may conclude that PEG is a good water marker ; it remains in the liquide phase whatever the physical conditions are, it does not penetrate food particles and is apparently not adsorbed by them.

TABLE 2

In vitro *effects of food hydration on calculated water space occupied by PEG-^{14}C in 5 trials*

	Control	Dehydrated (2)	Hydrated (3)
	9.857 (1)	9.612	13.933
	9.933	9.493	13.923
	9.929	9.608	13.817
	9.901	9.655	13.944
	10.024	9.657	13.912
Mean	9.929	9.605*	13.906
Theoretical volume	10	10	14

(1) Mean of two different counting vials.
(2) At 110°C for 24 hours.
(3) Two parts of cold water per one part of food.
* Different from the others ($P < 0.001$).

RADIO PEG BALANCE STUDY

Radio PEG was added in the drinking water (14173 dpm/ml), and daily water consumption as well as food consumption was measured. Four laying hens reached a steady state in a week and then feces were collected daily. Water content was estimated on an aliquot dried at 110°C overnight. Radioactivity was extracted with water and then counted in triplicate with a toluene-triton liquid scintillator. Feces collection lasted four consecutive days.

The results presented in table 3 show that the radioactivity recovered was 98.8 ± 2.25 p. 100 of that ingested the same day. In this experiment, feces were collected just at the beginning of the dark period, and a part of the activity drunk during the afternoon was excreted the next day. Thus, we compared the activity excreted on days (i) to that ingested on previous days (i — 1). In these conditions, the activity recovered was 100.9 ± 1.83 p. 100 of that ingested. Even though the balance study was better in the latter case, the recovery ratio did not vary from one in either of the conditions.

At the end of the experiment, plasma radioactivity was 1924 ± 53 dpm/ml and that of four control birds was 525 ± dpm/ml, *i.e.* about 4 times higher. Thus, some traces of the label enter the extracellular space.

TABLE 3

Results of the balance study on radioactive PEG ratio between cumulative activity in feces over cumulative activity ingested for four days

(in p. 100)

Hens	Feces and water from the same day	Feces of days i and water of days $(i - 1)$
1	93.7	96.1.
2	99.2	101.6
3	104.6	105.0
4	97.7	100.9
Mean	98.8 $\pm$ 2.26	100.9 $\pm$ 1.83

From measured water content and PEG concentration in feces, water consumption was calculated for each bird per day. Calculation showed water intake to be 185.5 $\pm$ 10.6 ml/day, while the measured consumption was 186.8 $\pm$ 7.2 ml/day. Thus, apparent water retention (intake-excreted) was 81.5 ml/day as compared to 81.7 ml/day.

We conclude that radioactive polyethyleneglycol in drinking water is a good marker for calculating water movements in the avian digestive tract.

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RÉSUMÉ

POLYÉTHYLÈNEGLYCOL RADIOACTIF : UN MARQUEUR DE L'EAU INTESTINALE CHEZ LES OISEAUX

Les marqueurs intestinaux utilisés chez les oiseaux transitent essentiellement avec la phase solide. L'étude des mouvements d'eau et d'ions dissous ne peut donc se faire avec de tels marqueurs. Nous avons donc essayé le polyethyleneglycol (PM = 4 000) marqué uniformément au ^{14}C.

In vitro ce marqueur reste toujours dans la phase liquide d'un mélange d'eau et d'aliment quelles que soient les conditions de pH (de pH 3 à pH 8) et d'hydratation de l'aliment. Même si de l'eau pénètre à l'intérieur des particules alimentaires, le PEG reste en solution.

In vivo le PEG est donné dans l'eau de boisson et on laisse les animaux se mettre en état d'équilibre pendant une semaine. A la suite de quoi, les bilans sont faits pendant quatre jours consécutifs sur quatre pondeuses. Lorsque l'on compare la quantité de PEG excrétée à celle ingérée le même jour, le taux de récupération est de 98,8 $\pm$ 2,25 p. 100. Mais si on compare la quantité excrétée à celle ingérée la veille, ce taux est de 100,9 $\pm$ 1,83 p. 100. Dans un cas comme dans l'autre le taux de récupération n'est jamais différent de 100 p. 100.

Le PEG radioactif est donc un bon marqueur de la phase liquide du contenu intestinal chez les oiseaux en état d'équilibre.