

## Relationship between small intestine transit and bile acid metabolism in axenic and holoxenic rats fed different diets

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**Summary.** The transit time of the small intestine was determined in 8 groups of rats differing in the presence or absence of intestinal flora, the presence or absence of lactose in the diet, and the mode of diet sterilization (autoclaving, irradiation). The irradiated diet was offered as paste and the autoclaved diet as pellets.

Transit was slower in axenic rats compared to holoxenic ones, and tended to be longer in rats fed the irradiated diet than in those fed the autoclaved one ( $p = 0.07$ ). Lactose had no effect.

When the values obtained for transit time were plotted either against those of the bile acid pool of the small intestine, or against those of the bile acid fecal excretion, a regression line was obtained.

The results suggested that transit time differences amply explained the larger bile acid pool and lower fecal excretion in axenic rats compared to holoxenic ones, as well as the larger pool and lower fecal excretion of rats fed the irradiated diet compared to the autoclaved diet.

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### Introduction.

Bile acid metabolism depends largely on the presence or absence of a bacterial flora in the digestive tract as well as on dietary factors. Variation in the bile acid pool of the small intestine from 1 to 4 and in bile acid fecal excretion from 1 to 2 have been previously observed in rats fed a semi-synthetic diet prepared and sterilized in different ways (Sacquet, Leprince and Riottot, 1979).

In order to investigate how diet modifications and the presence or the absence of bacterial flora induced these variations of bile acid metabolism, the transit time for the small intestine was studied. This paper describes how this transit time varied according to the afore-mentioned factors and demonstrates a double relationship between this time and the bile acid pool in the small intestine on the one hand, and between the same time and bile acid fecal excretion on the other.

## Material and methods.

The experiment consisted in determining the transit time of polyethylene glycol 4000, PEG, for the small intestine in rats placed in conditions similar, in every respect, to those of the abovementioned experiment studying bile acid metabolism. We then tried to establish whether a regression existed or not between this time and the bile acid pool of the small intestine, and between the same time and bile acid fecal excretion.

*Animals and diets.* — As in the bile acid experiment, 4-month old male inbred Fisher rats of our holoxenic and axenic breeding units were used. They were fed the experimental diets 1 month before transit time was studied. As previously, the basic diet included 220 g of casein, 580 g of maize starch, 90 g of maize oil, 50 g of cellulose, 45 g of a mineral and vitamin mixture. The diet was sterilized either by autoclaving or by irradiation at 4 megarads. When autoclaving was used, the diet was admixed with 200 g of water and pressed. For irradiation, no water was added, and it was introduced into polyethylene bags sealed under vacuum. The irradiated diet was given to the rats as a paste containing 50 p. 100 water. 10 p. 100 of lactose was added or not to the diet, and 8 experimental groups were constituted : GFI, GFLI, GFAu, GFLAu, CVI, CVLI, CVAu and CVLAu (GF = axenic or germfree, CV = holoxenic or conventional, I = irradiated diet, Au = autoclaved diet, L = 10 p. 100 lactose added).

### *Determination of transit time.*

1. — *Experimental procedure.* PEG-4000 (Merck) of the grade used for gas liquid chromatography and PEG <sup>14</sup>C-4000 (Amersham), with a specific activity of 55.6 mCi/mmol, were mixed with saline to obtain a 5 mM solution containing 0.05  $\mu$ Ci per ml.

The night before the test, food was removed from the cages and the rats were only allowed to drink water. After they had been slightly anesthetized with ether and carefully given 4 ml of the PEG solution through a Silastic (Dow Corning) stomach tube, they were returned to their cages. They were killed by cervical dislocation under slight ether anesthesia at 15, 30, 60, 90, 120, 180, 240 and 360 min after the stomach intubation. *Post-mortem* samples were taken rapidly : clamps were placed on the stomach-esophageal junction, the pylorus and the ileo-cecal junction. The organs (stomach, small intestine, whole cecum and large intestine together) were cut off, introduced into vials (containing 25 ml of distilled water for the first two organs and 50 ml for the third unit), weighed and homogenized with an ultra Turrax grinder (Janke and Kunkel KG, 7813 Staufen i. Br., Germany).

An aliquot of this suspension was centrifuged at 20 000 g for 15 min, and 1 ml of the supernatant was introduced into a vial containing 15 ml of a 30 p. 100 Triton  $\times$  100 (Merck) scintillation liquid composed of : POPOP 0.1 g, PPO 4 g, Toluene 1 l. Radioactivity was measured in a SL 30 Intertechnique Spectrometer with an external standard device. The recovered radioactivity represented 90 to 95 p. 100 of that administered to the rat.

TABLE 1

Means  $\pm$  standard deviations of parameters <sup>(1)</sup>  $P_1$ ,  $P_2$ ,  $P_3$ , input  $t_1$ , output  $t_2$  and transit half-times  $T$  <sup>(2)</sup> for the small intestine in the experimental groups

Group <sup>(3)</sup>	CVAu	CVLAU	CVI	CVLI	GFAU	GFLAU	GFI	GFLI
Number of rats	22	16	16	16	33	16	20	17
$P_1$ .....	$0.029 \pm 0.002$	$0.028 \pm 0.003$	$0.024 \pm 0.003$	$0.032 \pm 0.003$	$0.015 \pm 0.001$	$0.018 \pm 0.001$	$0.014 \pm 0.001$	$0.022 \pm 0.003$
$P_2$ .....	$90.9 \pm 8.3$	$80.2 \pm 5.3$	$113.8 \pm 9.1$	$107.1 \pm 3.8$	$103.7 \pm 6.1$	$118.4 \pm 4.7$	$126.0 \pm 7.1$	$116.7 \pm 13.4$
$P_3$ .....	$0.026 \pm 0.006$	$0.020 \pm 0.005$	$0.026 \pm 0.008$	$0.032 \pm 0.007$	$0.009 \pm 0.001$	$0.014 \pm 0.002$	$0.009 \pm 0.001$	$0.065 \pm 0.001$
$t_1$ .....	$23.3 \pm 1.3$	$24.4 \pm 3.0$	$29.0 \pm 3.7$	$21.8 \pm 1.9$	$45.9 \pm 3.5$	$37.8 \pm 1.7$	$49.8 \pm 3.6$	$31.2 \pm 4.9$
$t_2$ .....	$117.4 \pm 12.3$	$115.0 \pm 11.9$	$140.6 \pm 17.5$	$128.4 \pm 7.2$	$176.6 \pm 11.6$	$168.3 \pm 9.4$	$204.7 \pm 13.2$	$223.7 \pm 35.4$
$T$ .....	$94.1 \pm 12.4$	$90.7 \pm 12.3$	$111.7 \pm 17.9$	$106.6 \pm 7.5$	$130.8 \pm 12.2$	$130.8 \pm 9.4$	$154.9 \pm 13.7$	$192.5 \pm 35.7$

<sup>(1)</sup> Parameters of the equations of input  $t = -\frac{1}{P_1} \ln \left( 1 - \frac{Y_1}{100} \right)$ , where  $Y_1$  is the percentage of radioactivity which left the stomach, and  $t = P_2 - \frac{1}{P_3} \ln \left( 1 - \frac{Y_2}{100} \right)$ , where  $Y_2$  is the percentage of radioactivity which entered the caecum and large intestine.

<sup>(2)</sup> Times of  $P_2$ ,  $t_1$ ,  $t_2$ ,  $T$  in minutes and  $P_1$  and  $P_3$  in minutes<sup>-1</sup>.

<sup>(3)</sup> CV = holoxenic rat ; Au = autoclaved diet ; L = lactose-containing diet ; T = irradiated diet ; GF = axenic rat.

The percentage of radioactivity found in the organs at different times after stomach intubation was noted using 4 to 6 rats for each transit time determination.

2. — *Mathematical treatment of experimental data.* The distribution of radioactivity in the organs at different times was calculated as follows :

$y_1$  = p. 100 of recovered radioactivity which left the stomach, i.e. PEG input into the small intestine,

$y_2$  = p. 100 of recovered radioactivity which entered the cecum and large intestine, i.e. PEG output from the small intestine. Experimental values fitted with the following equations :

$$y_1 = 100 [1 - e^{-P_1 t}] \quad (1)$$

$$y_2 = 100 [1 - e^{-P_3(t-P_2)}] \quad (2)$$

where  $t$  is the time in minutes, and  $P_1$ ,  $P_2$  and  $P_3$  are the parameters computed from the experimental values. The fitting technique for these two models was based on maximization of the related likelihood of the parameter (or parameters) of the model. This likelihood function was computed from the data, supposing that the errors in the observed percentages were distributed normally and independently. This assumption led to standard minimization of the sum of the squares of deviations between the observed and the estimated values for the  $y$  variable. The standard errors of the parameter estimates,  $P_1$ ,  $P_2$  and  $P_3$ , were obtained by linearization of the model near the optimum reached by the maximization process in the parametric space. The correlation matrix for the parameter estimates in equation 2 was deduced from the reciprocal of Fisher's information matrix estimated at the solution. The values were fitted using the maximum likelihood program of Ross (1970).

Equations 1 and 2 were used to determine the times of the 50 p. 100 input and the 50 p. 100 output of PEG for the small intestine or input half-time,  $t_1$ , and output half-time,  $t_2$ . The difference between these times was the transit half-time, or  $T$ , for the organ.

*Statistical analysis.* — Axenic versus holoxenic rats, irradiated versus autoclaved diets and lactose-free versus lactose-containing diets were compared using a two-way variance analysis (Snedecor and Cochran, 1957).

## Results.

Table 1 presents the results on PEG transit in the small intestine : estimates and standard deviations of the parameters  $P_1$ ,  $P_2$ ,  $P_3$ , input  $t_1$ , output  $t_2$  and transit half-times  $T$ . The variability of  $T$  was small, and its estimates were used in variance analysis (table 2). The presence of an intestinal flora very significantly reduced  $t_1$ ,  $t_2$  and  $T$ . Lactose did not produce any significant change. The irradiated diets, compared to the autoclaved, did not modify  $t_1$  but significantly increased  $t_2$  ( $p = 0.05$ ) ;  $T$  appeared to increase, although the variation only approached the level of significance ( $p = 0.07$ ).

When the values of  $T$  in the different groups were plotted against those of the small intestine bile acid pools in the previous experiment (Sacquet, Leprince and Riottot, 1979), a regression line was obtained :  $r = 0.90$ ,  $p < 0.01$  ;  $Y = 0.66 T - 29.5$ , where the bile acid pool,  $Y$ , is expressed in  $\mu$ moles per 100 g of body weight and half-time  $T$  in minutes (fig. 1). The slope of the line (0.66) indicates that  $Y$  increased

TABLE 2  
Variance analysis

	Input $t_1$		Output $t_2$		Transit half-time T	
	F	P	F	P	F	P
Presence vs absence of bacterial flora.....	28.4	0.025	52.7	0.001	20.2	0.02
Irradiated vs autoclaved diets ..	0.001	—	11.2	0.05	7.8	0.07
Presence vs absence of dietary lactose .....	4.1	0.14	0.02	—	0.5	—

Number of degrees of freedom of treatment = 1.

Number of degrees of freedom of error = 3.

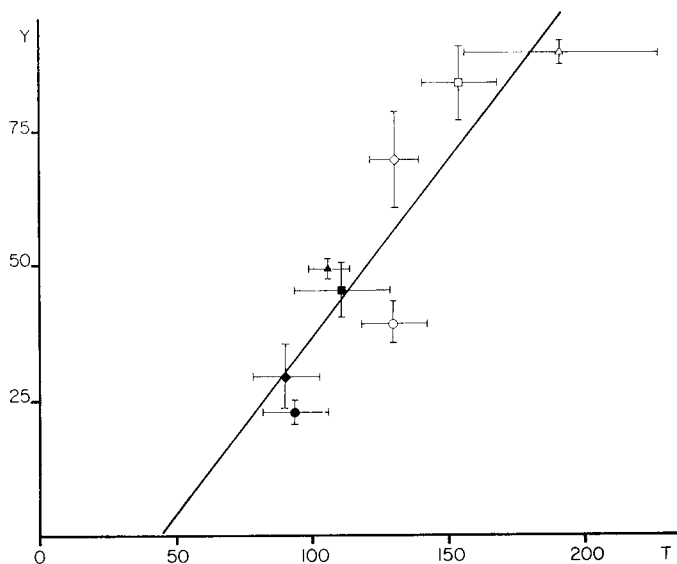


FIG. 1. — Relationship between small intestine bile acid pools Y and transit half-time T of PEG 4000

○ GFAu    □ GFI    ◇ GFLAu    △ GFLI  
● CVAu    ■ CVI    ◆ CVLAu    ▲ CVLI

Ordinate = Y expressed in  $\mu\text{moles}/100$  g of body weight.

Abscissa = transit half-time T expressed in minutes.

The symbols represent the arithmetic means and the bars the standard deviation.

very rapidly according to  $T$ . Similarly, a regression line was obtained between  $T$  and bile acid fecal excretion :  $r = 0.87$  ;  $Y = -0.073 T + 18.7$ , where  $Y$  is bile acid fecal excretion expressed in  $\mu\text{moles}/100 \text{ g}$  of body weight/day (fig. 2).

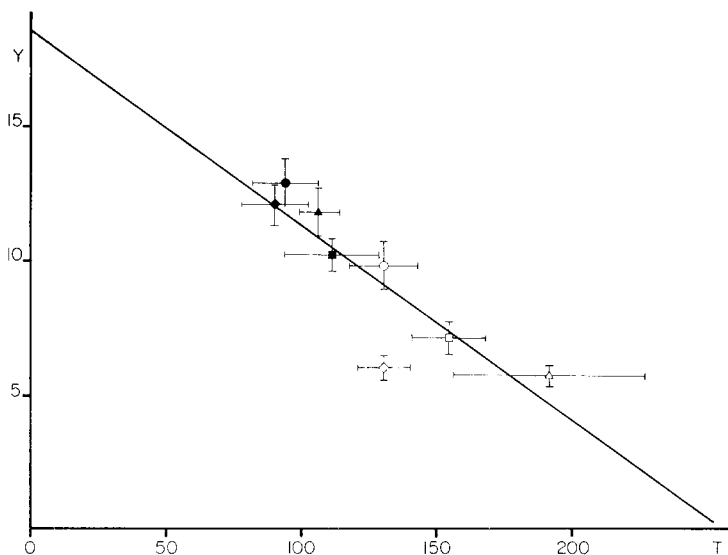


FIG. 2. — Relationship between bile acid fecal excretion and transit half-time  $T$  of PEG 4000  
Ordinate =  $Y$  is expressed in  $\mu\text{moles}/100 \text{ g}$  of body weight/day.  
Other symbols as in figure 1.

## Discussion.

Measuring small intestine transit time in rats presents theoretical and practical difficulties. Many techniques have been tried with different markers, modes of administration and methods of evaluating marker propulsion through the intestine (Goodman, Lewis and Schuck, 1952 ; Goodman *et al.*, 1952 ; Marcus and Lengemann, 1962 ; Summers, Kent and Osborne, 1970 ; Nilsson and Johansson, 1973 ; Poulakos and Kent, 1973 ; Purdon and Bass, 1973). Some authors used as a criterion the percentage of intestinal length travelled by the marker front after a specified time ; others noted the position of 75, 50 and 25 p. 100 of the marker. Still others determined the distribution of the marker at different times after it was administered. Goodman *et al.* (1952) were the only authors to conceive a mathematical approach to transit determination, but it has never been used by other authors. The arrival in the colon of the front of a marker, lactulose, has been used as a criterion in recent studies on humans (Duane and Hanson, 1978 ; Hardison, Tomaszexski and Grundy, 1979). The presence of this unabsorbable carbohydrate in the large intestine is conveniently detected by the technique of Bond and Levitt (1975). The time necessary for the marker to first appear in the cecum is defined in our work as  $P_2$ . Previous studies (Marcus and Lengemann, 1962 ; Poulakos and Kent, 1973) suggested that this time differs from half-time  $T$  or longer times. Differences between  $T$  and  $P_2$  are found in 3 of our 8 experimental groups

(GFAu, GFI, GFLI). When  $P_2$  was used instead of T, the relationship between transit time and bile acid pool persisted ( $r = 0.91$ ), but the equation of the regression line was very different ( $Y = 1.39 P_2 - 95.5$ ) and the differences in transit time between rats fed the irradiated diets and those fed the autoclaved diet were reduced. Also, the equation of the regression between transit time and bile acid fecal excretion became :  $r = 0.80$ ,  $Y = -0.15 P_2 + 25.5$ .

The choice of a marker is another source of difficulty because the transit time observed varies with the marker : when bacterial spores were used as a marker in rats of the same breed fed the Au diet, transit time was longer than in the present experiment (Sacquet, Garnier and Raibaud, 1970). PEG was probably a better marker than solid particles because bile salts, as PEG, are very readily soluble in water. This marker, however, has not the amphipatic properties of bile salts, which are soluble in both water and lipids, and it cannot thus be affirmed that PEG and bile salts transit rates are the same.

A third point of controversy is that transit was studied while the rats were fasting. This was justified since rats do not have a gall-bladder and their enterohepatic circulation is continuous. Therefore, only diet-induced modifications of transit, persisting beyond digestion periods, are liable to act on the bile acid pool. However, the present study is incomplete : We do not know the extent to which the transit rate is modified by the diet during digestion periods. According to Marcus and Lengemann, T is longer when the rats are fed than when they are starved.

In spite of these limitations, our work suggests the following conclusions : (i) the presence of an intestinal flora decreased T in the small intestine, (ii) the rats accustomed to eat the irradiated diet tended to have a longer transit time T than those fed the autoclaved diet, (iii) there was a relationship between T and the bile acid pool of the small intestine, as well as between T and bile acid fecal excretion.

The first observation confirms the results obtained in mice by Abrams and Bishop (1967) and in rats by Sacquet, Garnier and Raibaud (1970). The slower transit rate in axenic rodents is also found in other species : « abnormal » electromyographic records have been obtained in axenic calves (Dardillat *et al.*, 1977). How the intestinal flora exerts this effect remains nearly unknown, for no studies have been published since Strandberg *et al.* (1966) established that cecal strips from axenic rats did not respond to biologically active amines in the same way as those from holoxenic animals.

The second observation inclines us to think that intestinal motility during fasting depends on the diet the rats are accustomed to. If so, it may be possible to condition intestinal motility by dietary factors. Which of these factors are involved remains to be determined, since the Au and the I diets differed not only in the mode of sterilization, but in the physical form (pellets, paste) in which they were offered.

The third result agrees with the hypothesis we have recently proposed, i.e. that the main characteristics of bile acid metabolism (intestinal pool and fecal excretion) largely depend on small intestine transit time (Sacquet, Leprince and Riottot, 1979). The lower fecal excretion and the larger bile acid pool of axenic rats compared to holoxenic ones has been observed by several authors (Kellog, 1971, 1974 ; Wostmann, 1973 ; Sacquet *et al.*, 1975, 1977a). However, no explanation of this difference is available. Several hypotheses are feasible, e.g. there is a lower basal metabolism in axenic rats (Desplaces, Zagury and Sacquet, 1963 ; Wostmann, Bruckner-Kardoss

and Knight, 1968 ; Levenson *et al.*, 1969) and therefore a decrease in many oxidative processes, such as the conversion of cholesterol into bile salts ; in axenic rats there is no bacterial transformation of bile salts into less absorbable, and therefore more readily excreted molecules (Sacquet *et al.*, 1977b). However, our study suggests a simple explanation : slower transit results in a larger pool, which induces a greater flow of bile acids through the liver and a decreased biosynthesis by feed-back regulation. Further work is needed to confirm this hypothesis.

Slower transit may also help to explain the larger bile acid pool in rats fed the irradiated diet as compared to those fed the autoclaved one. On the contrary, animals fed the Au and LAu diets exhibited similar transit times, but the latter had larger pools than the former. It is possible that these increased pools proceed from the greater bile acid ileal absorption observed in rats fed the LAu diet (Riottot *et al.*, 1977). Variation in transit rate is not the only factor modifying the bile acid pool.

Transit time itself depends on factors other than intestinal flora and diet. Duane and Hanson (1978) observed that humans staying in a metabolic ward for 2 to 3 weeks exhibited very wide differences in transit time. They showed a significant correlation between that time and bile acid pool ( $r = 0.69$ ). Thus, this relationship has been reported both in a species with a gallbladder and in one without. Whether dietary variations modify the bile acid pool in humans as it does in rats deserves to be investigated.

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**Résumé.** Le temps de transit au niveau de l'intestin grêle est déterminé dans 8 groupes de rats qui diffèrent par la présence ou l'absence de flore intestinale, la présence ou l'absence de lactose dans l'aliment, le mode de stérilisation de l'aliment (autoclavage ou irradiation). En outre, l'aliment irradié est donné sous forme de pâte, et l'aliment autoclavé sous forme de comprimés.

Le transit est plus lent chez les rats axéniques que chez les holoxéniques ; il tend à être plus lent chez les rats qui reçoivent l'aliment irradié que chez ceux qui reçoivent l'aliment autoclavé ( $p = 0.07$ ). Le lactose alimentaire est sans effet.

Une régression linéaire est observée entre le temps de transit au niveau de l'intestin grêle, et la quantité d'acides biliaires présente au niveau de cet organe, ainsi qu'entre ce temps de transit T et l'excrétion fécale des acides biliaires.

Ces résultats suggèrent que les différences observées entre les pools intestinaux et les excréments fécaux d'acides biliaires sous l'action soit de l'aliment soit de la présence ou de l'absence de flore relèvent, au moins en partie, des variations que ces facteurs impriment à la vitesse du transit au niveau de l'intestin grêle.

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