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In situ collection of intestinal lymph in the non-restrained rat

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Introduction

Lipophilic nutrients are generally absorbed in the enterocytes of the small intestine where they are incorporated in chylomicrons transporting these substances via the intestinal lymph to the general circulation (*Blomhoff et al.* 1984, *Tso & Simmonds* 1984). Of the *in vivo* systems used to study the digestion, absorption and transport of lipids, the lymph fistula rat model has been one of the most commonly used (*Tso & Simmonds* 1984). This model was first introduced by *Bollman et al.* (1948), who also devised a restraint cage for postoperative care (*Bollman* 1948). In addition to the lymph fistula model there are several other *in vivo* as well as *in vitro* models described for studying intestinal absorption (*Tso & Simmonds* 1984). Most of these methods do not involve restraint of conscious cannulated animals. However, they are all limited to certain part(s) of the absorption process. The stress caused by restraint has been shown to cause gastric ulcers in rats (*Bonfils et al.* 1960) and also to alter vitamin A status (*Morita & Nakano* 1982). From an ethical point of view the use of restrained animals is undesirable.

Environmental contaminants, such as chlorinated dioxins, have been shown to inhibit the normal hepatic vitamin A storage in different animal species (*Hanberg et al.* 1990, *Håkansson & Ahlborg* 1985). This effect could be due to impaired intestinal absorption of dietary vitamin A. As an effect of dioxin might occur at any step in the absorption process, this issue has to be studied in a model covering the entire process of intestinal absorption; from the course of events in the intestinal lumen until the retinoid is transferred into the lymph. The lymph fistula rat model was considered the most appropriate model to investigate this problem.

The purpose of this study was to develop a technique for collection of intestinal lymph in the conscious non-restrained rat. The method reported here was used to study the absorption of vitamin A, but may also be used for studying other lipophilic substances absorbed via the lymphatic system.

Materials and methods

Experimental animals

All procedures involving animals were approved by the local ethical committee on animal experiments. Conventional male Sprague-Dawley rats were obtained from ALAB Laboratorietjänst (Sollentuna, Sweden). Since the main purpose of this study was to investigate the effect of an oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the intestinal absorption of retinol (*Hanberg et al.*, in preparation) the animals were housed two in each wire bottomed cage prior to operation. Illumination cycle (12 h light, 12 h dark) was automatically controlled. Room temperature was maintained at 20 to 21° C with a relative humidity of 50 %. Prior to the start of the study, the animals were acclimatized for one week and were provided food (R3, EWOS AB, Södertälje, Sweden) and tap water *ad libitum*. At the start of the study, six rats were given corn oil (2.5 ml/kg) via gavage (being vehicle controls to TCDD-treated rats). After the corn oil administration, the rats were pairfed to the TCDD-treated rats and received 25 g feed per rat per day. Five days after corn oil administration the intesti-

nal lymph duct was cannulated. After the operation the rats received less food (Table 1) according to the consumption of the TCDD-treated operated rats. In addition, two groups of six rats were similarly treated, except for the anaesthesia and lymph duct cannulation (nonoperated rats). One of these groups was pairfed to the operated rats (i.e. to the TCDD-treated operated rats) and the other group was fed *ad libitum*. The body weight of the rats was 270 ± 8 g at the start of the study (day of corn oil administration).

Surgical procedure

The rats were fasted overnight. In the morning, 30 minutes prior to operation, 1 ml of a cream/milk mixture (20 % fat) was administered via gavage in order to make the intestinal lymphatic vessel become more prominent, with a milky colour. The rats were anaesthetized with a mixture of Hypnorm (10 mg fluanisonum/ml, 0.2 mg fentanylum/ml, Janssen Pharmaceutica, Beerse, Belgium), Dormicum (5 mg midazolam/ml, Roche, Basel, Switzerland), and water (1:1:2, 3 ml/kg, i.p.). Prior to the operation, Atropin (0.05 mg/kg, ACO, Solna, Sweden) was given subcutaneously to reduce excessive bronchial secretion. The fur around the trunk was removed in order to later to be able to fix the lymph collecting tube with adhesive tape (Fixomull stretch, Beiersdorf, Finland).

The rat was placed on its back on an electric heating pad and a transverse incision was made across the abdomen about 10 mm posterior to the costal border. The liver was retracted upward towards the right, and intestines and stomach displaced to the left to expose the superior mesenteric artery and the intestinal lymphatic vessel. The liver and the intestine were covered by gauze moistened with Normal saline (37° C). Thin ligaments between the liver and the intestine were cut and the intestinal lymph duct was freed from connective tissue. If necessary, the lymph flow in the duct was increased by carefully massaging the small intestine with the operator's fingertips. In order to prevent the catheter (Intramedic PE50, Clay Adams, Parsippany, N. J., USA) from being displaced from the duct with movement after the operation, the catheter was formed as a spiral (Figure 1), which made it more flexible. The catheter was cut obliquely in a way to produce a short bevel placed to point upwards and was filled with saline. The duct was perforated approximately 5 mm left of vena cava using a 0.6 mm angled hypodermic needle. The catheter was inserted 1–2 mm into the duct (Figure 1) and the free end of the catheter was placed below the level of the animal. After making sure that the lymph was flowing smoothly into the catheter, it was secured in the duct with 1–2 drops of tissue adhesive (Histoacryl, B Braun Melsungen AG, Germany), which was allowed to set (1–2 min) before the gauze was removed and the internal organs were returned to their normal anatomical positions and wetted with tempered saline. The peritoneum, and subsequently the skin, were sutured (4-0 Novafil CE-4 suture, Davis+Geck, UK), with the catheter protruding in the middle of the wound. The stitches were fixed with Histoacryl.

The rat was given another dose of Atropin, as well as an intramuscular dose of Narcanti (0.1 mg/kg, Du Pont, Hertfordshire, UK) to antagonize the anaesthetic. The catheter was led through the perforated screw cap of a tube (Nalgene cryoware 2.0 ml, Nalge Company, N. Y., USA), and cut to appropriate length. The trunk of the rat was tied with adhesive tape, and the cap was fixed with tape on the upper abdomen. The tube was screwed onto the cap and fixed with tape in a position which permitted the rat to move about (Figure 2). The rat was allowed to wake up in a cage bedded with cellulose wadding, underneath an infra-red bulb in order to gently recover from surgery.

Experimental design

After a 24-hour-recovery from surgery (or at the corresponding time-point for nonopera-

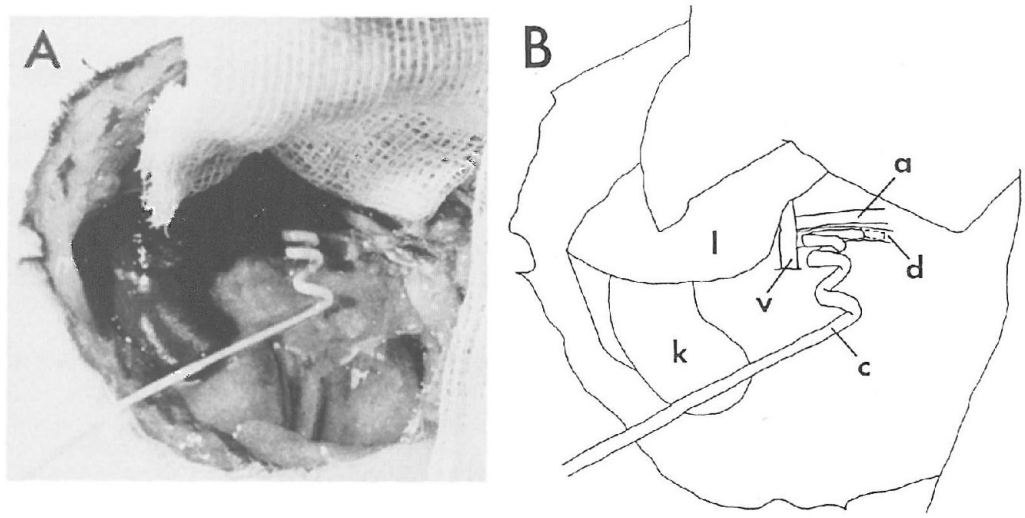

Figure 1. Photography (A) and drawing (B) of the abdominal cavity (liver (l); right kidney (k); superior mesenteric artery (α); vena cava (v)) showing the catheter (c) inserted into the main intestinal lymphatic duct (d). Note the spiral form of the catheter.

Figure 2. Photograph of a rat with the intestinal lymph duct cannulated and the collecting tube attached to the abdomen with Fixomull tape. Note that the rat is free to move while lymph is collected.

ted rats), 6 operated and 12 nonoperated (pairfed and *ad libitum* fed) rats were given a dose of ^{3}H-retinol in corn oil (0.05 nmol, 2.5 μCi, 0.5 ml/rat) via gavage. The rats were placed in metabolic cages in order to collect urine and faeces. The lymph of the operated rats was collected for 24 hours. The tubes were changed every second hour for 12 hours and after 24 hours when the rats were killed by blood withdrawal under anaesthesia (Mebumal, Nordvacc, Skärholmen, Sweden; 90 mg/kg, i.p.). Liver, kidneys, lungs and thymus were removed and rinsed in saline before weighing. The absorption, distribution and elemination of ^{3}H-retinol will be reported elsewhere (*Hanberg et al.*, in preparation).

Statistical procedures

The body and organ weights of the operated rats were compared with those of both the *ad libitum* fed and the pairfed nonoperated rats using Student's *t*-test (SigmaStat Statistical software, Jandel Scientific, Erkrath, Germany). Statistical comparisons were considered significant at the $p < 0.05$ level. Values for body and organ weights, food consumption and amount of lymph are expressed as mean ± standard deviation (SD) for each group.

Results

After recovering from anaesthesia, operated rats were observed eating pelleted diet, drinking water and moving about in their cages, as well as performing grooming routines. To get an overall assessment of the condition of the operated rats, body weight gain and organ weights were compared with those of the nonoperated rats (Table 1). Compared to the *ad libitum* fed nonoperated rats, the operated rats lost weight instead of gaining weight. The absolute weights of liver and thymus were lower in the operated rats. The absolute weights of kidneys and lungs were lower, while the relative weights were higher than in the *ad libitum* fed nonoperated rats. Due to the fact that the operated rats were pairfed to TCDD-exposed rats they had a considerably lower food consumption than the *ad libitum* fed rats (Table 1). Therefore, they were also compared to pairfed nonoperated rats. The reduced intake was found to account for the differences in the weights of kidney, lung and thymus. The food restriction was, however, only

Table 1. Body and organ weights and food consumption for *ad libitum* fed nonoperated rats, pairfed nonoperated rats and pairfed operated rats.

Parameter	*Ad libitum* fed nonoperated rats	Pairfed[1] nonoperated rats	Pairfed[1] operated rats
Food consumption[2] (g)	46 ± 2	5 ± 0	5 ± 1[a]
Body weight gain/loss[2] (g)	13.2 ± 4.1	−18.1 ± 1.4	−34.7 ± 4.2[a,b]
Liver weight (g)	12.3 ± 1.1	6.73 ± 0.37	8.37 ± 0.33[a,b]
Relative liver weight (g/100 g bw)	4.03 ± 0.32	2.80 ± 0.09	3.63 ± 0.16[a,b]
Kidney weight (g)	2.21 ± 0.16	1.90 ± 0.10	1.85 ± 0.13[a]
Relative kidney weight (g/100 g bw)	0.73 ± 0.04	0.79 ± 0.04	0.80 ± 0.05[a]
Lung weight (g)	1.14 ± 0.03	1.22 ± 0.26	1.07 ± 0.04[a]
Relative lung weight (g/100 g bw)	0.38 ± 0.01	0.51 ± 0.14	0.46 ± 0.01[a]
Thymus weight (g)	0.69 ± 0.05	0.46 ± 0.07	0.44 ± 0.07[a]
Relative thymus weight (g/100 g bw)	0.23 ± 0.02	0.19 ± 0.03	0.19 ± 0.03[a]

[1] Rats were pairfed to TCDD-exposed operated rats (reported elsewhere).
[2] Measured for the two days from operation (or for nonoperated rats the corresponding day) until termination.
[a] Statistically significantly different ($p < 0.05$) from *ad libitum* fed nonoperated rats.
[b] Statistically significantly different ($p < 0.05$) from pairfed nonoperated rats.

partly the cause of the body weight loss. The liver weight of the operated rats was higher compared to the pairfed nonoperated rats.

The lymph duct cannulated rats produced on average 0.5 ± 0.2 ml intestinal lymph per hour for the first 12 hours. For the period 12–24 hours the pooled lymph was 1.4 ± 0.5 ml, i.e. a considerably lower flow rate (0.1 ± 0.0 ml/h). For the first six time periods (0–12 h) lymph was successfully collected for all six rats. However, at the last period (12–24 h) one of the rats gnawed a hole in its collecting tube and the lymph was lost.

Discussion

In the present study, the rats were pairfed to TCDD-treated rats and therefore, the food consumption could not be used as an estimate of the general condition of the rats. The operated rats lost more weight than the pairfed nonoperated rats. In the operated rats, mean postoperative body weight was 87 % of mean preoperative weight (two days), compared to 93 % in pairfed nonoperated rats and 104 % in *ad libitum* fed nonoperated rats. This is in accordance with the results of *Toriumi et al.* (1994), who reported a postoperative body weight of 84.4 % for rats with bile and pancreatic fistulas and 106.9 % for nonoperated *ad libitum* fed controls (four days). The pairfeeding in our study (11 % of *ad libitum* consumption postoperatively) was thus only partly responsible for the reduced body weight. Body weight loss is, however, generally considered as a manifestation of stress-response (*Takase et al.* 1992). Several factors have been reported to be involved in postoperative weight loss (*Toriumi et al.* 1994). Besides reduced nutrient and fluid intake during recovery, the catabolic state induced by the trauma and stress of surgery contributes to the weight loss. In addition, postsurgical pain has been suggested to contribute to the body weight loss (*Liles & Flecknell* 1993).

The initial high amount of lymph collected (0.5 ± 0.2 g/h) is probably due to the corn oil administration and the subsequent lower lymph flow (0.1 ± 0.0 g/h) probably reflects the low food intake of the rats during this period. The volume of lymph obtained has been reported to vary with the dietary and fluid balance of the animal (*Bollman et al.* 1948). For rats receiving a mixed diet and water as desired, approximately 20 ml of intestinal lymph was reported to be collected every 24 h (0.8 ml/h). *Lambert* (1965) reported an intestinal lymph flow of 0.5–1.0 ml/h in the fasting animal and of 2.5 ml/h during the digestive period. An average lymph flow of 0.60 ml/h during 24 h collection was reported for rats cannulated in the mesenteric lymph duct (*Ribaya-Mercado et al.* 1988). Our data on intestinal lymph flow of non-restrained rats are thus in accordance with previously reported data.

The method described here is useful for collecting lymph for 12–24 hours, which in most cases is sufficient for following absorption of single oral doses of lipophilic substances. During the last collection period (12–24 h), one of six operated rats started to gnaw on its tube. Therefore, when studying absorption for more than 12–24 h the rats have to be kept under more frequent observation. The most important advantage with the described method is the enhanced ability for the animals to move within their cages, which presumably reduces stress. Accordingly, the absorption process will be more physiological than if conventional methods for lymph collection are used. In addition, this method makes the lymph collection in rats more ethically justified as the rats do not have to be restrained. However, postsurgical analgesia should be provided since pain might have contributed to the postsurgical weight loss (*Liles & Flecknell* 1993).

In conclusion, we have developed a method for lymph collection in the non-restrained rat. The method, which allows almost physiological conditions, is suitable for studying absorption of lipophilic substances *in vivo* in a more humane way than previous methods used.

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Summary
A technique for collection of intestinal lymph in non-restrained rats has been developed. The lymph is continuously collected in a tube attached to the abdomen of the rat. This technique allows the rat to move about freely in the cage during lymph collection and is therefore both more physiological and more humane than the Bollman cage system of near-total restraint.

Sammanfattning
En metod för samling av intestinal lymfa hos råttor utan rörelseinskränkning har utvecklats. Lymfan samlas kontinuerligt i ett rör på råttans mage. Denna metod tillåter att råttan rör sig fritt i buren under den tid lymfa samlas och metoden är därför både mer fysiologisk och mer etiskt försvarbar än de metoder där råttan är mer eller mindre förhindrad att röra sig.

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