J. Anim. Physiol. a. Anim. Nutr. 68 (1992), 146–150
© 1992 Verlag Paul Parey, Hamburg und Berlin
ISSN 0931-2439

Receipt of Ms. 15. 4. 92

Department of Animal Physiology, Warsaw Agricultural University, Warsaw, Poland

A model for long-term sampling of lymph from the jejunal lymphatic trunk in pigs and sheep

By R. Zabielski and S. G. Pierzynowski

Introduction

Studies performed upon lymph formation and absorption of molecules from the gastrointestinal tract to the lymph often require the use of selectively catheterized smaller lymphatic vessels, e. g. hepatic or intestinal ones (Lascelles and Morris 1961). The application of acute experiments for this purpose is controversial, since the anethesia was found to depress the lymph flow approximately three times (Shannon and Lascelles 1968 b). Owing to this, preparations of animals for long-term study, although more difficult than in acute trial, seem to enable the investigation of lymph in conditions much closer to physiological ones. In most earlier described surgical methods for a long-term study, authors have cannulated major lymphatics, i. e. the thoracic duct, and without reinstalling lymph between collections (Kiriyama et al. 1988, Rampone 1959). Loss of lymph could be prevented by reintroducing it either into the common jugular vein, employing a lymphatico-venous shunt (Lascelles and Morris 1961, Shannon and Lascelles 1967) or into the oesophagus (Willamson and Sells 1986). Lymph from the gastrointestinal tract was also collected selectively from lymphatics of smaller size, i. e. hepatic trunk, ileal trunk or lumbar trunk in sheep (Heath 1964, Lascelles and Morris 1961), in calves (Shannon and Lascelles 1968 a, b) and in pigs (Binns and Hall 1966), anyhow it was often mentioned that the abate, and soon thereafter the lack of flow, was a major problem disturbing satisfactory function of the catheter.

The aim of present study was to propose a model (swine and ovine) for long-term lymph sampling from the jejunal lymphatic trunk (*truncus lymphaticus jejunalis*, Schummer et al. 1981). In our model the mentioned problems concerning lymph outflow between collections were solved by returning lymph into the posterior vena cava. The description of pig's preparation has been emphasised, as to our knowledge, pigs were not explored intensively. In the presented report, feeding effect in pigs and intraduodenal (i. d.) rape oil infusion in sheep on lymph outflow from the intestine were estimated. Moreover, the above method has been used for the investigation of dietary cholesterol absorption in chronic experiments on conscious piglets (Bartnikowska and Zabielski 1990).

Material and methods

Animals. Five Great White Polish pigs, both sexes (15.5–21 kg) and five Polish Merino ewes (20–24 kg) were used in the present experiment. Animals were held in the individual pens throughout the entire experimental period. Piglets were fed twice a day, with the standard mixture of fodder (0.4 kg/day) consisting of 15.5% protein and 2.5% fat. Sheep were fed with meadow hay ad libitum. After 7 days of adaptation to experimental conditions, the surgery was performed to implant catheters into the jejunal lymphatic trunk and into the posterior vena cava, in sheep additionally the duodenum was cannulated.

Catheters. Catheters were constructed of silicone tubing 1 × 0.5 mm in diameter for piglets, and 1.5 × 0.8 mm in diameter for sheep (Silastic, Dow-Corning Corp., Midland, Michigan, USA), and all the remained preparings were made similarly for both animal species. The catheter for the lymphatic trunk (25 cm long) was supported with a double silicone cuff mounted on 6 and 8 mm far from the inserted top to protect against a catheter sliding out of the lymphatic vessel. Additionally, a silicone ring (10 mm diameter) glued in the two-thirds far from the inserted portion was used for stabilizing the catheter between the abdominal muscles. The catheter for the posterior vena cava (35 cm long) had similar cuffs and rings, although the cuffs were placed about 20 cm from the top and corrected during surgery if necessary.

Surgery. Piglets were fasted for 12 h before surgery, while sheep for 24 h. Two hours before anesthesia, animals received 0.2 l of cream mixed with 2 g of instant coffee, this procedure enlarged and enabled easier identification of lymphatic vessels in the visceral region during laparotomy. Premedication was administered by intramuscular injection of 2% xylazine (0.05 ml/kg body weight; Rompun, Bayer, Germany). Surgery was performed in the aseptic conditions, under general anesthesia induced with xylazine and maintained thereafter with intravenous administration of pentobarbiturate (15 mg/kg; Vetbutal, Polfa, Poland). Dextral dorsoparacostal laparotomy (15 cm long) was made to access the small intestine. Between loops of the small bowel, held by an assistant using gauze sponges or drapes rinsed with saline, the cranial root of the mesentery and the cranial mesenteric artery with its characteristic conjunctions, the two colonic arteries were searched for (in pigs). The jejunal lymphatic trunk could be seen as lymphatic originated of numerous small-size lymphatic vessels draining a chain of lymph nodes along the jejunum, and to some extend distal duodenum, and a part of ileum. In the mesojejunum the jejunal lymphatic trunk ran together with easy to recognize the cranial mesenteric artery. In sheep the jejunal lymphatic trunk had to be differentiated from located cranially the lymphatic hepatic trunk. In the upper part of the mesentery the jejunal lymphatic trunk was carefully detached below its conjunction with the colonic lymphatic trunk for a length of about 15 mm. After supporting with two silk ligatures (4-0; Ethicon Inc., Somerville, NJ, USA) and incising the trunk, the catheter was introduced against the lymph flow to a depth of 4 to 6 mm and fixed. The valves were destructed by a fine tweezers or a hypodermic needle, when necessary. Additionally, the catheter was fastened to the mesentery with the help of another 2 or 3 silk ligatures. Afterwards, the posterior vena cava was exposed, and about 3 to 4 cm caudal to the liver diaphragm a purse-string silk (6-0) suture (3 to 4 mm in diameter) was prepared on it. The vessel wall was then incised in the middle of the suture and the second catheter was inserted in the direction of the heart. The tip of catheter was positioned where the largest suctioning effect was observed, then the position of both cuffs was corrected, and the catheter fixed by purse-string suture and one additional suture to surrounding tissues. Two free ends of both catheters were immediately connected with the help of a silicone joint. Such prepared venous catheter served as a "biological pump", slightly aspirating lymph from the catheterized lymphatic trunk and also turning it back into the systemic blood circulation. Laparotomy was routinely closed and both catheters were led out through the surgical wound with protective rings placed between sutured muscles. Antibiotic, benzylpenicillin (Penicyllinum procainicum, Polfa, Poland) was administered for one week at 15,000 IU/kg per day. The catheters were controlled three times a day and the venous catheter was flushed with heparin solution (10 U/ml Hep Lock, Elkins-Sinn Inc., NJ, USA).

Collection of lymph

All collections were performed without immobilizing the animals and in their own boxes. Animals were previously accustomed to a frequent visiting their cages for sampling. Collections were made from the fourth day after surgery. During collecting lymph the venous

catheter was disconnected, filled with heparin solution and sealed. The free end of lymph catheter was connected to a polyethylene vial attached by the adhesive tape to the right flank of animal. Every time the vial filled with lymph, it was emptied by syringe, and the contents was pooled into heparinized plastic tubes held in ice. In such manner the lymph flow was monitored in piglets in preprandial (1 h) and postprandial conditions (7 h). In sheep the lymph flow was measured in both, basal conditions (although without food and water restriction), during 3 h lasting intraduodenal infusion of 0.9% NaCl (3 ml/h/kg) and graded doses of the rape oil (0.07, 0.14 and 0.42 g/kg), and after the infusion (2 h). The volume of lymph (ml/h/kg) was expressed as averages (mean, S.E.) and statistical analysis was done using the Student-t test.

Results and discussion

All animals quickly recovered after surgery and appeared to be clinically normal in the time of experiment. Piglets lost average of 0.8 kg due to surgery (sheep approximately 2 kg). Animals recovered their weights within 3–4 days, afterwards piglets gradually increased their weight, while the weight of sheep remained stable. When the experiment terminated, in general anesthesia the right side laparotomy was repeated in pigs at 12th day and in sheep at 21st day after the first surgery. Lymphatics in the mesenery and position of implanted catheters were examined with the help of 1% Paris Blue solution, afterwards all catheters were remowed. No signs of formed collateral circulation or dilatation of catheterized trunk were observed.

In piglets, the flow from the jejunal lymphatic trunk before the morning meal was 0.30 ± 0.06 ml/h/kg and feeding increased markedly this outflow (Fig. 1) peaking at 2 h postprandially at 0.75 ± 0.08 ml/h/kg and returned to the basal values 4 h after food consumption. The outflow from the jejunal trunk seemed, however, to be much smaller comparing with cannulations of major lymphatics in pigs, i.e. the intestinal trunk or the thoracic duct reported by BINNS and HALL (1966), or the thoracic duct in newborn piglets (KIRIYAMA et al. 1988). This could suggest proportionally bigger share of ileal, large intestine and hepatic lymph than jejunal lymph in the pig thoracic duct.

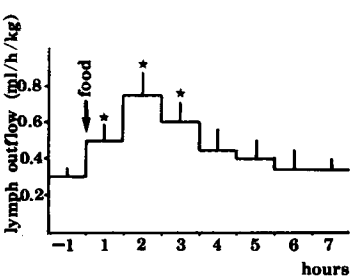

Fig. 1. The jejunal lymph outflow (ml/h/kg) in piglets ($n = 5$; mean ± S.E.) before and after feeding; arrow: feeding time; stars indicate the significant difference ($P < 0.05$)

In sheep the basal outflow from the jejunal lymphatic trunk was 0.75 ± 0.05 ml/h/kg and the intraduodenal infusion of saline as well as rape oil stimulated the outflow in a dose-dependent manner (Tab. 1). In case of 0.9% NaCl and first two doses of rape oil (0.07 and 0.14 g/kg) the highest lymph outflow was observed during 2 h postinfusion collection (2.5, 3.1 and 4.4 ml/h/kg respectively). The highest lymph outflow (4.75 ml/h/kg) was already seen during infusion of the highest dose (0.42 g/kg) of rape oil. Stimulation of lymph outflow by 0.9% NaCl i.d. infusion suggest participation of jejunal mechanoreceptors in the regulation of lymph flow in sheep. Similarly to pig, there was no available data concerning outflow from the jejunal trunk in sheep to compare, although presented results

are consistent with the average lymph outflow reported from the intestinal trunk in sheep (LASCELLES and MORRIS 1961). However, we did not observe so wide variation of flow being from 20 to 120 ml/h in the intestinal lymphatics, as noted in the study cited above.

Table 1. **The jejunal lymph outflow (ml/h/kg body weight) in basal conditions (30 min), during 3 h infusion (3 ml/h/kg) of saline or graded doses of rape oil and during 2 h after the infusion from sheep (n = 5)**

Treatment	Basal	Infusion	After Infsuion
Saline	0.80 ± 0.10^{a}	1.25 ± 0.15^{b}	2.15 ± 0.20^{c}
Rape oil			
(g/kg) 0.07	0.70 ± 0.05^{a}	1.10 ± 0.20^{b}	3.10 ± 0.40^{d}
0.14	0.75 ± 0.05^{a}	1.95 ± 0.35^{c}	4.40 ± 0.65^{e}
0.42	0.75 ± 0.10^{a}	4.75 ± 0.40^{e}	3.40 ± 0.40^{de}

Mean ± S.E. Different letters given with the results indicate the significant difference ($P < 0.05$)

Authors describing procedures of cannulation for study upon lymph flow often showed numerous technical difficulties with maintaining implanted catheters in sheep (HEATH 1964) and in dog (RAMPONE 1959), whereas in pigs their abrupt behaviour was particularly pointed out (BINNS and HALL 1966). This later could be avoided by previous accustoming animals to the experimental protocol. For our experiment pigs as well as sheep were not discomforted by immobilizing them during whole time of collection. Silicone rings and cuffs glued on the catheters additionally protected them against pulling out. Surgical model presented here comparing with that for catheterization of intestinal trunk in sheep (LASCELLES and MORRIS 1961) was simpler, the whole procedure of pancreas dissection and extensive preparing were avoided, although the drained area in our model was limited to the jejunum, a part of distal duodenum and a part of ileum. HEATH (1964) remarked that the collateral circulation arises faster when the flow of lymph is stopped in the vessel by its occlusion from outside, it may also appear when clots would cork the lumen of catheter, in order to avoid the slowed flow and the adhesion of clots on the tubings, we forced the flow in the lymphatic catheter. For this purpose as well as for resumption of lymph we utilized the negative pressure occuring in the thoracic fragment of posterior vena cava serving as "biological aspirating-pump".

Summary

A model for long-term collection of lymph from the small intestine on pigs and sheep was presented. During surgery a silicone catheter was implanted into the jejunal trunk *(truncus lymphaticus jejunalis)*, additionally, a second catheter was inserted into the posterior vena cava towards the heart to such a depth as to obtain the highest suckling effect. Both catheters were connected immediately to enable the reintroduction of jejunal lymph into the systemic blood circulation. The negative pressure present in the posterior vena cava in its thoracic fragment was found to improve the lymph flow through the catheter inserted into the jejunal trunk. Preprandial outflow of lymph from the jejunum in pigs was 0.3 ml/h/kg body weight and increased up to 0.78 ml/h/kg 2 hours after feeding. Lymph outflow in sheep in basal conditions was 0.75 ml/h/kg and increased in dose dependent manner after rape oil intraduodenal (i.d.) infusion up to 4.75 ml/h/kg. However, mechanical stimulation of the intestinal receptors by 0.9% NaCl also stimulated lymph flow.

Zusammenfassung

Eine Methode zur langfristigen Lymphsammlung aus der jejunalen Lymphbahn bei Schweinen und Schafen

Es wird eine Methode zur langfristigen Lymphsammlung im Dünndarmbereich von Schweinen und Schafen vorgestellt. Mit Hilfe eines chirurgischen Eingriffs wurde ein Silikon-Katheder im

truncus lymphaticus jejunalis implantiert. Zusätzlich wurde ein zweiter Katheter in die hintere vena cava in Richtung Herz eingesetzt. Beide Katheter wurden sofort miteinander verbunden, um die Wiedereinführung der jejunalen Lymphe in den Blutkreislauf zu gewährleisten. Der negative Druck in der hinteren vena cava im Thoraxteil förderte den Lymphfluß durch das Katheter im truncus lymphaticus jejunalis. Der preprandiale Lymphfluß des Jejunums betrug bei Schweinen 0,3 ml/h/kg Körpergewicht und stieg postprandial auf 0,78 ml/h/kg an. Beim Schaf betrug der Basiswert 0,75 ml/h/kg. Er stieg nach intraduodenaler Infusion von Rapsöl auf 4,75 ml/h/kg. Eine mechanische Stimulation der intestinalen Receptoren durch 0,9% NaCl-Lösung stimulierte ebenfalls den Lymphfluß.

References

Bartnikowska, E.; Zabielski, R., 1990: J. Anim. Physiol. a. Anim. Nutr. *64*, 174–179.
Binns, R. M.; Hall, J. G., 1966: Brit. J. Exp. Pathol. *47*, 275–280.
Heath, T., 1964: Am. J. Anat. *115*, 569–580.
Kiriyama, H.; Harada, E.; Syuto, B., 1988: Nutr. Rep. Int. *37*, 779–784.
Lascelles, A. K.; Morris, B., 1961: Quart. J. Exp. Physiol. *46*, 199–205.
Rampone, A. J., 1959: J. Appl. Physiol. *14*, 150–152.
Vollmerhaus, B., 1981: In: Nickel, R.; Schummer, A.; Seiferle, E. (Eds.): The anatomy of the domestic animals, Vol. 3, Berlin und Hamburg: Verlag Paul Parey, 292–440.
Shannon, A. D.; Lascelles, A. K., 1967: Aust. J. Biol. Sci. *20*, 669.
–; –, 1968a: Quart. J. Exp. Physiol. *53*, 194–205.
–; –, 1968b: Quart. J. Exp. Physiol. *53*, 415–421.
Willamson, E. P. M.; Sells, R. A., 1986: Transplantation *42*, 136–140.

Author's address: R. Zabielski, Rakuno Gakuen University, Department of Veterinary Medicine, Ebetsu, Hokkaido, Japan 069