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STARCH MICROSPHERE INDUCED SMALL INTESTINAL ISCHAEMIA

Blood flow and morphologic investigations of late effects

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The small intestine is very sensitive to ionizing radiation and may thus be severely injured by the high radiation doses necessary to control the growth of abdominal tumours. It is possible to protect the intestine against radiation injury by inducing temporary ischaemia and hypoxia by arteriolar embolization of degradable starch microspheres (FORSBERG et coll. 1978, 1979). This procedure induces a transient, selective, and severe ischaemia throughout the small intestine (LOTE et coll. 1980) without causing detectable acute vascular injury or thrombosis (LOTE 1981 a, b). Possible late adverse effects were investigated in cats and the results are now reported.

Material and Methods

Animal preparation. Fifteen adult cats of either sex weighing from 1.7 to 4.5 kg (mean 2.4 kg) were used. The animals were fed on water only the last 24 hours before the experiment. The cats were anaesthetized with sodium pentobarbital (40 mg/kg). A butterfly needle was inserted into a femoral vein, and a saline infusion (10 ml/kg/h) was established. Two surgical procedures separated by an interval of 14 days were performed on each animal. On day 1, a midline laparotomy was performed. An electromagnetic probe was positioned over the main trunk of the superior mesenteric artery. After isolation and ligation of the distal end of the right colic branch of the superior mesenteric artery, a cannula (OD 0.63 mm) was inserted, advanced 3 to 5 mm in the proximal direction, and secured in this position with the cannula tip pointing into the main trunk of the superior mesenteric artery. Degradable starch microspheres were injected through this cannula. Care was taken to secure that the cannula tip and the electromagnetic flow probe were separated by at least 2 cm. Following induction of temporary small intestinal ischaemia by intraarterial injection of starch microspheres, the intraarterial cannula was removed, the artery ligated, and the abdomen closed by Dexon sutures. On day 14, each animal was reoperated during pentobarbital anaesthesia. Plastic cannulas were inserted into the abdominal aorta through both femoral arteries. Another cannula was inserted into the left ventricle of the heart through the right carotid artery. Correct catheter position was confirmed by recording left ventricular pressures before and after tissue flow determinations by carbonized microspheres injected into the left ventricle. The cats were killed by intracardiac injection of potassium chloride. The abdomen was not reopened ante mortem.

Tissue blood flow. Carbonized microspheres (New England Nuclear, Boston, MA, USA) with diameter of 14.71.2 μm and labelled with ^{113}Sn or ^{141}Ce were used to measure blood flow per g of tissue. The microspheres were suspended in 10% Dextran with addition of Tween-80 to prevent aggre-

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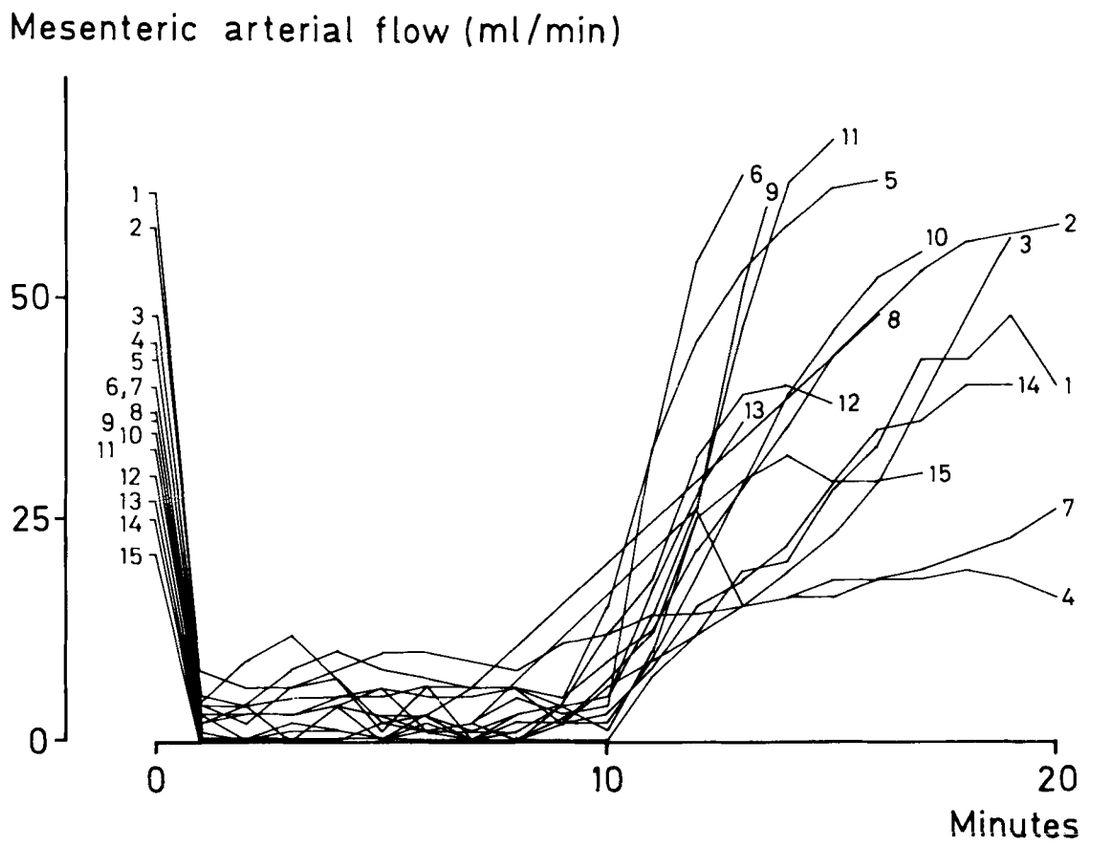


Fig. 1. Blood flow (electromagnetic) in the superior mesenteric artery before, during, and following mesenteric arteriolar embolization of degradable starch microspheres in 15 cats.

gation. The sonified suspensions contained about 1.2×10^6 spheres/ml and were mixed thouroughly immediately before injection of 1.0 ml suspension into the left ventricle. Simultaneously, a reference blood sample was withdrawn from the distal aorta at a constant rate as described by HEYMANN et coll. (1977) and confirmed in the cat by SEGADAL & SVANES (1979). Net activity in blood and tissue samples was determined in a multichannel gamma spectrometer (ICN Instruments SC 722, Oakland, CA, USA) and the tissue flow was calculated from the following formula: QT=QR × (cpm T/cpm R), where QT is blood flow rate in tissue sample, QR is blood flow rate in aortic reference sample, cpm T is counts/min/g in tissue, and cpm R is counts/min in reference blood sample. During injection of carbonized microspheres, the haemodynamic state of the animals was carefully monitored. Isolated ventricular ectopic beats were occasionally observed in 3 of 15 animals, without any significant influence on the arterial blood pressure.

Tissue samples. Small intestinal tissue samples were taken at 9 consecutive levels 10 to 90 cm from the ligament of Treitz. The mucosa/submucosa layer was separated from the muscularis layer by simple stripping. Microscopic examination confirmed that the mucosa samples consisted of lamina propria, muscularis mucosae, and stratum submucosum. The muscularis layer contained only smooth muscle and serosal lining. Samples for histopathologic examination were also taken at 10, 25, 50, 75 and 90 cm distance from the ligament of Treitz, fixed in 4% neutral formalin, and processed for paraffin embedding. Routinely cut sections were stained with hematoxylin and eosin and periodic acid Schiff (PAS) stains. The microscopic slides were examined for signs of small intestinal inflammatory reactions and starch microsphere remnants in the tissue, and granulomatous intestinal inflammation (STERNLIEB et coll. 1977).

Arterial blood flow. The blood flow in the superior mesenteric artery was recorded by an electro-

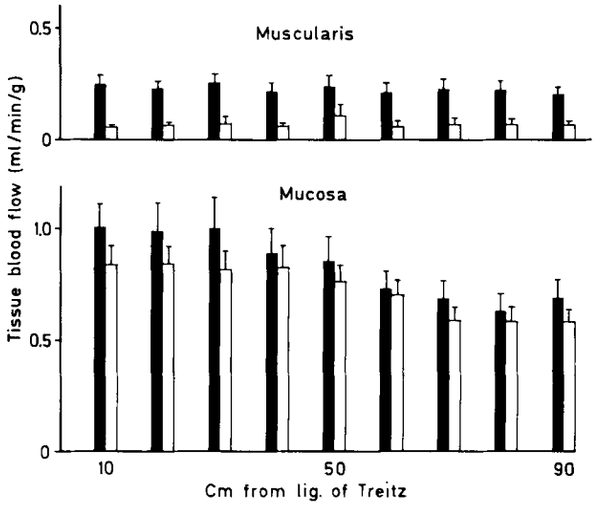


Fig. 2. Small intestinal blood flow (carbonized microsphere distribution) measured during laparotomy in 16 cats (■) compared with blood flow measured 14 days after temporary starch microsphere induced ischaemia (□) in 15 cats. Mean and SEM of observations obtained at different distances aborally from the ligament of Treitz are given.

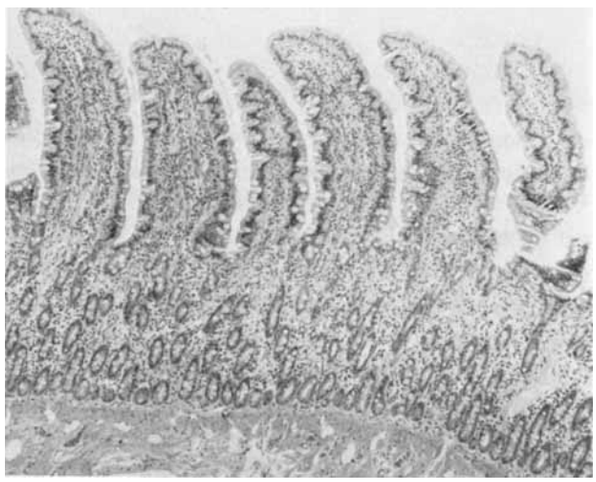


Fig. 3. Small intestinal biopsy showing lamina propria and muscularis mucosae 14 days after temporary starch microsphere induced ischaemia. Well preserved structures, normal villi, glandular crypts, and absence of inflammatory changes. H+E×36.

magnetic flow probe positioned over the artery and connected to a square wave flowmeter (Carolina Medical Electronics, 501, King, NC, USA). The zero point was established by brief occlusion of the vessel distal to the probe. Aortic blood pressure and pulse rate were determined with a Statham pressure transducer P23De connected to a Hewlett-Packard 7758 A recorder calibrated directly against a sphygmomanometer.

Control animals. The tissue blood flow to the small intestinal mucosa and muscularis was compared with that observed in 16 other animals which underwent similar surgical preparation (LOTE et coll. 1980, LOTE 1981 a). In the control animals, the tissue blood flow was determined immediately after the surgical procedure on day 1, and before induction of temporary intestinal ischaemia. In the present series, however, intestinal tissue flow was determined 14 days after induction of temporary ischaemia, and before the abdomen was reopened.

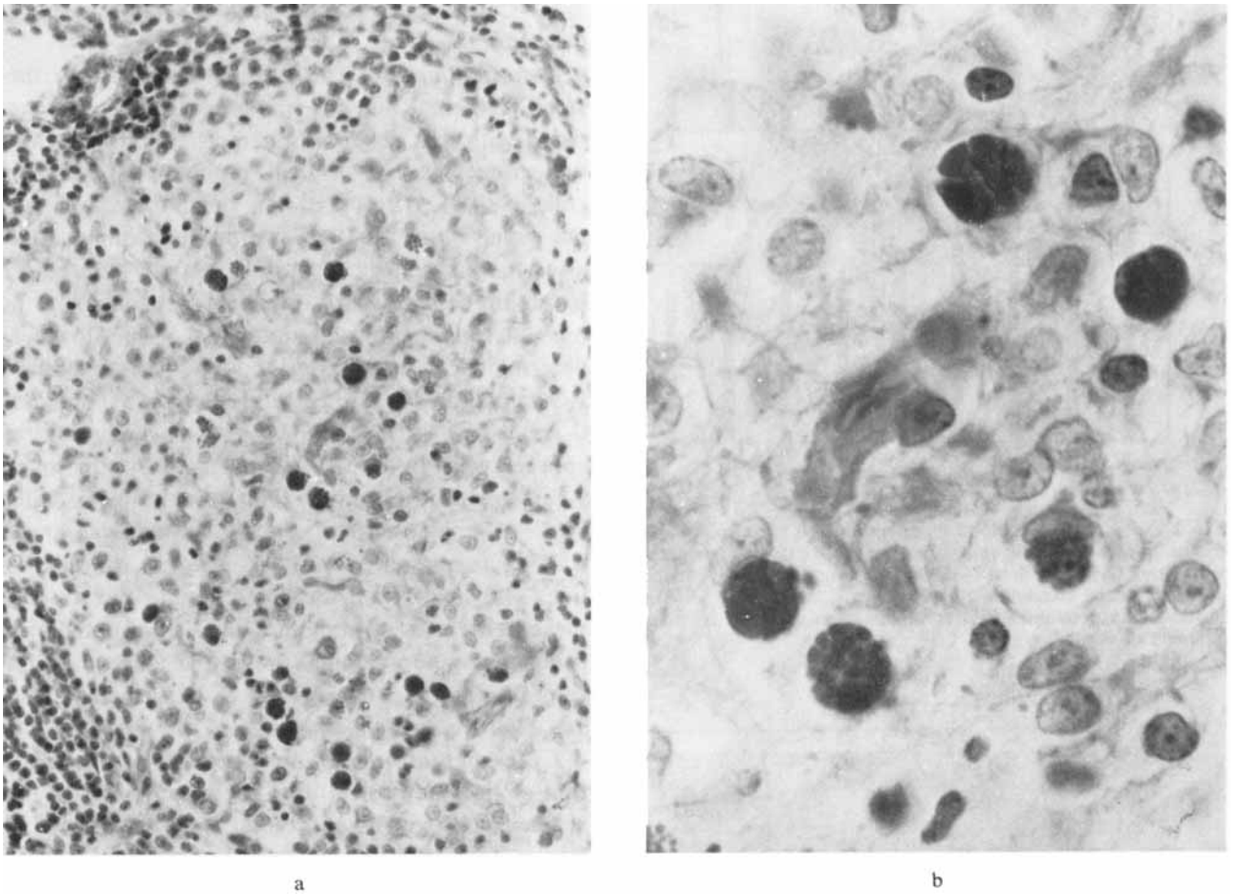
Degradable starch microspheres. The starch spheres were produced and supplied by Pharmacia AB, Uppsala, Sweden (Pharmacia 40/15, batch 70635, 60 mg/ml). Mean sphere diameter was 40 μm. The microspheres were suspended in isotonic saline. The suspension was injected into the superior mesenteric artery through the indwelling catheter to produce temporary small intestinal ischaemia. Serum amylase was determined as described by ZINTERHOFER et coll. (1973).

Experimental procedure. After surgical preparation of the animals on day 1, control superior mesenteric electromagnetic blood flow was recorded. Serum for amylase determinations was obtained. The microsphere suspension was then injected into the superior mesenteric artery at a rate of 2.0 ml/min until arterial flow stabilized at a minimum level, usually 0 to 10 ml/min. Additional suspension was injected as required to keep the superior mesenteric aterial flow at this minimum level for 10 min. The microsphere injection was then terminated. Arterial flow was monitored for 20 min or until control flow was regained. The abdomen was then closed and the animal permitted to recover. At surgery on day 14, a single carbonized microsphere blood flow determination was performed before the animal was killed.

Statistics. Student's two-tailed t-test for comparison of two means was used to test statistical probability. A p-value less than 0.05 was regarded as statistically significant.

Results

Superior mesenteric arterial blood flow on day 1. The variations of mesenteric arterial blood flow is illustrated in Fig. 1. The flow was immediately reduced to very low levels by starch sphere embolization; it was reduced from an average of 39 ml/min to



a b

Fig. 4. a) Germinal centre of an ileal Peyer's patch showing engulfed starch particles residing within macrophages 14 days after starch microsphere induced ischaemia. PAS-stain × 250. b) Same section at higher magnification. Several large starch containing macrophages. PAS-stain × 1 000.

2.5 ml/min within one min. This severe ischaemia was easily maintained for 10 min by additional starch sphere administration as required. Ischaemia reversed itself within 5 to 10 min upon termination of starch microsphere injection. Two animals (Nos 4, 7) had a more protracted recovery period. All animals recovered without complications from anaesthesia, surgery, and temporary starch microsphere induced small intestinal ischaemia. No intraabdominal infections occurred. The group of cats gained weight from 2.4 kg (range 1.7–4.5 kg) to 2.5 kg (range 2.0–4.1 kg) on day 14.

Serum amylase activity. Mean serum amylase activity was 1365 units (range 2280–820 units). Normal range in humans by this method of analysis is 40 to 140 units.

Small intestinal tissue blood flow on day 14. The tissue blood flow in small intestinal mucosa and muscularis layers 14 days after severe temporary starch microsphere induced ischaemia is given in Fig. 2. Compared with the control group, the mucosal blood flow determined at 9 gut levels in animals exposed to starch microspheres was slightly lower. However, the difference was not statistically significant ($p>0.05$) at any gut level. In contrast, blood flow in the muscularis layer was significantly lower in animals exposed to starch microsphere embolization ($p<0.01$). The two animals (Nos 4, 7) which made slow recoveries from the induced ischaemia gained weight (0.1 kg each) during the observation period, and showed tissue flow values after 14 days above the group average.

Morphologic examination on day 14. Macroscopically the gut appeared completely normal in all animals. Microscopic examination of small intestinal tissue obtained at 5 different levels from the jejunum to the terminal ileum did not demonstrate signs of ischaemic tissue injury or acute or chronic intestinal

inflammation in any of the 15 animals (Fig. 3). Starch microspheres or starch remnants were not observed in the vessels or the interstitial tissue of the mucosa, submocusa, or muscularis layers. However, PAS-positive fragments were located within macrophages residing in germinal centres of ileal Peyer's patches, and could be demonstrated in all 15 animals 14 days after starch microsphere embolization (Fig. 4). No obvious correlation between the degree of starch sphere persistence and serum amylase level was found, nor any obvious correlation between the duration of microsphere induced ischaemia and starch fragment persistence. No inflammation or cellular reactive changes were observed around starch containing macrophages within Peyer's patches in the ileum.

Discussion

Mesenteric embolization of degradable starch microspheres induced an immediate and severe small intestinal ischaemia which was maintained for 10 min before the superior mesenteric arterial blood flow was allowed to recover. In all but 2 animals, the superior mesenteric arterial flow rapidly recovered upon termination of the starch microsphere injection. The delayed blood flow restoration observed in these 2 animals did not cause late ischaemic injury to the small intestine.

Mucosal tissue flow determined 14 days after the temporary intestinal ischaemia was not significantly different from that of the control group, and was comparable to previous results obtained in the cat (GRANGER et coll. 1980). In contrast, muscularis blood flow was lower. Admittedly, the control and experimental groups are not quite comparable since the small intestinal blood flow was determined during actual laparotomy in the former, and before relaparotomy in the latter group. Probably the observed muscularis and mucosa tissue blood flow differences are explained by intestinal vascular inflammatory dilatation in response to laparotomy, and not by starch microsphere induced ischaemic injury to the muscularis layer, since all animals tolerated the ischaemia well. Thirteen of fifteen animals increased in weight during the observation period. Obviously, the blood flow to all wall layers after the temporary ischaemia was adequate to sustain normal intestinal function.

Intraperitoneal starch may induce starch granulomas after a latence period of 1 to 2 weeks (STERNLIEB et coll.). No evidence of such granulomas was observed at microscopic examination of small intestinal tissue massively exposed to intravascular starch microspheres 14 days previously. However, small amounts of sphere remnants and starch fragments engulfed by macrophages could still be demonstrated in lymphoid tissue in the ileum. Reexamination of microscopic slides obtained 120 to 135 min after starch microsphere embolization (LOTE 1981 b) showed that all visible starch fragments at that time were phagocytosed by macrophages located outside the capillaries. There is strong evidence in favour of the assumption that the intracellular PAS-positive material represents sphere remnants. The present experiments do not elucidate the intermediate steps of sphere degradation and phagocytosis. However, there is evidence from human material that the PAS-positive fragments are transported into lymphoid tissue by macrophages (LOTE et coll., unpublished observations). Thus, starch microspheres may be eliminated from the circulation in two ways, by enzymatic degradation and by fragmentation and phagocytosis. Whether the phagocytosis takes place intravascularly or extravascularly cannot be elucidated by the present findings. Clearly, starch fragments within macrophages are metabolized very slowly compared with the rapid elimination of intravascular starch spheres. The absence of inflammation in the vicinity of starch loaded macrophages strongly indicate that no injury was elicited by such intracellular starch persistence.

In summary, mesenteric embolization of degradable starch microspheres instantly induced severe temporary small intestinal ischaemia which did not cause detectable late adverse effects in the small intestine of cats. Starch fragments persisted for 14 days within macrophages located in intestinal lymphoid tissue. Such starch persistence did not cause intestinal injury or inflammation.

SUMMARY

Severe temporary small intestinal ischaemia was induced in 15 cats by mesenteric embolization of degradable starch microspheres. After an observation period of 14 days, small intestinal histopathology and tissue blood flow were investigated. Intracellular starch fragments were found in tissue macrophages in ileal Peyer's patches in all animals, but no late adverse effects of starch microsphere embolization were observed.

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