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# Magnetic granules: a novel system for specific drug delivery to esophageal mucosa in oral administration

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## Summary

Presuming an application to targeting therapy for esophageal cancer, magnetic granules containing ultrafine ferrite ($\gamma$-$Fe_2O_3$), brilliant blue FCF, and bioadhesive polymers (10 : 1 : 9 w/w) were prepared, and their properties as a drug carrier were examined in vitro and in vivo. When 5 mg of granules containing a mixture of hydroxypropyl cellulose (HPC) and Carbopol 934® (6 : 4 w/w) was flushed into an agar-gel tube with 20 ml of 0.65% HPC solution, about 90% of the granules were held in the region of the applied magnetic field (about 1700 G). Holding of the magnetic granules was influenced by the viscosity of the solution, magnitude of the magnetic field and the ferrite content of granules. The granules were administered to rabbits with about 2 ml of 0.65% HPC solution via catheter without anesthesia. Approximately the entire amount of granules was held in the region at 2 h after administration with magnetic guidance for the initial 2 min. The results indicate that the new targeting system presented might be useful for local chemotherapy of esophageal cancer and also for other diseases of the alimentary canal.

## Introduction

Site-specific delivery of chemotherapeutic agents is one of the major approaches used to increase bioavailability and to reduce systemic side effects. Recently, several types of magnetically guided drug carriers for targeting therapy have been proposed, such as albumin microspheres (Widder et al., 1978, 1981; Morimoto et al., 1980, 1981; Sugibayashi et al., 1982), emulsions (Akimoto and Morimoto, 1983; Morimoto et al., 1983; Akimoto et al., 1985) and liposomes (Kiwada et al., 1986). Controlled localization of drug carriers has been difficult to achieve, because intravascular administration of such carriers resulted in their uptake by the reticuloendothelial system. Therefore, biodegradable drug carriers with 'magnetic properties' have been developed as described above. Magnetic guidance of intravascular particles by an externally applied magnetic field has been proven to be feasible. Ibrahim et al. (1983) reported the localization of magnetic, submicroscopic and biodegradable nanoparticles in the kidney and liver after intravenous administration using samarium permanent magnets. How-

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ever, there was no report concerning the application of magnetic guidance in oral dosage forms.

Esophageal cancer occurs less frequently than stomach cancer; however, the recovery ratio and the survival ratio in the case of esophageal cancer are still low. Three types of treatment are currently employed for esophageal cancer, i.e., surgical excision, radiotherapy, and chemotherapy. However, esophageal cancer is often found to be in a progressed state, combined therapy being therefore conducted in general. Also, systemic chemotherapy often causes the appearance of side effects.

Therefore, with the intention of improving the topical effects of anticancer drugs, we initiated the present investigation of magnetic guidance applied for local targeting chemotherapy of esophageal cancer by oral administration, a new route in the field of magnetic guidance. In this study, granules were prepared consisting of ultrafine ferrite, bioadhesive polymers, and brilliant blue FCF as model anticancer drug. Firstly, the capacity for holding of the granules by a magnetic force was determined in vitro. The effects of some conditions, e.g., viscosity of the solution used with the granules, premagnetization time of the granules, strength of the magnetic field, and ferrite content in the granules, on the holding ability were examined. Subsequently, targeting efficiency and fixation of the granules in rabbit esophagus were also examined.

## Materials and Methods

*Chemicals*

Ultrafine ferrite ($\gamma$-$Fe_2O_3$, needles of 0.01–0.05 $\mu$m diameter and 0.1–0.5 $\mu$m length) was kindly supplied by Dainichi Seika Color & Chemicals Mgf. Co., Ltd. Brilliant blue FCF (B.B.) and powdered carrageenan were purchased from Tokyo Kasei Kogyo. Carboxymethylcellulose sodium (CMC-Na), hydroxypropylmethyl cellulose (HPMC), sodium polyacrylate (PAA-Na) and agar powder were purchased from Wako Pure Chemical Industries. Hydroxypropyl cellulose-H (HPC) and carboxyvinyl polymer (Carbopol 934®, CP) were obtained from Nihon Soda and B.F. Goodrich, respectively. Powdered tamarind gum was a gift from Professor Prakongpan (Mahidol University, Thailand). Powdered tragacanth and sodium alginate (AA-Na) were from Japanese Pharmacopoeia XI. Pentobarbital sodium injection (Nembutal® injection) and 1% lidocaine injection (Xylocaine® injection; with epinephrine, 1 : 100000) were purchased from Dainabot and Fujisawa Pharmaceutical, respectively. All other reagents were of special reagent grade.

*Preparation of magnetic granules*

Brilliant blue FCF, ultrafine ferrite, and bioadhesive polymers (1 : 10 : 9 w/w) were mixed with ethanol or purified water and allowed to stand overnight. After drying the mixture at 70 °C for 2 h, it was granulated by passage through a 20-mesh sieve. The particles obtained were then dried overnight in vacuo, and granules which passed through a 20-mesh sieve but remained on a 50-mesh sieve were used as samples.

*Determination of brilliant blue FCF content in granules*

Brilliant blue FCF (B.B.) was extracted from the granules by stirring in ethanol for 2 h followed by centrifugation at 3000 rpm for 10 min. The amount of B.B. in the supernatant was determined spectrophotometrically at 626 nm using a Hitachi 124 spectrophotometer.

*Selection of bioadhesive polymers for preparation of granules*

Granules containing HPC/CP mixture in the ratio 6 : 4 w/w, CMC-Na, HPMC, powdered tragacanth, powdered tamarind gum, powdered carrageenan, PAA-Na, or AA-Na, were prepared as described previously without combination of ferrite.

The release patterns of B.B. from each granule were determined by the agar-gel bed method (Machida et al., 1979). An agar-gel used was prepared by pouring a saline solution containing 1% agar (30 ml) into a petri dish, upon which a stainless-steel ring (25 mm, i.d.) was placed. A 10 mg sample of granules was scattered within the ring and incubation at 37 ± 1 °C was performed after removal of the ring. At appropriate intervals,

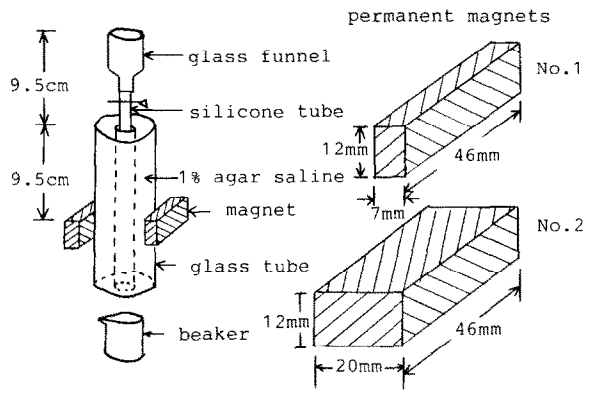


Fig. 1. Illustration of the apparatus and the permanent magnets used for the holding test.

the remaining granules on the bed were washed out with a small amount of purified water. The bed was then homogenized using 100 ml of purified water at 10000 rpm for 5 min followed by centrifugation at 3000 rpm for 10 min. The amount of B.B. in the supernatant was determined spectrophotometrically as described above.

Separately, a 10 mg sample of the same granules was stirred in 50 ml of purified water for 2 h, homogenized with 50 ml of purified water and the same amount of agar-gel bed, and centrifuged similarly. The amount of B.B. determined in the supernatant was regarded as the value at 100% release of B.B. for calculation of the release ratio for each period.

*Evaluation of targeting ability in vitro using model esophagus*

The apparatus used in this experiment is illustrated in Fig. 1. A tubular agar gel (5 mm i.d., 20 mm o.d., 15 cm length) as a model of the esophagus was prepared with 1% agar in saline in a glass tube, and positioned vertically. Two permanent magnets (Sumitsu & Co.) were positioned on the tube as shown in Fig. 1. A pair of magnets, No. 1 (Sm-Co magnet) or No. 2 (neodymium-iron-boron magnet), could generate a magnetic field of about 700 or 1700 G, respectively, on the internal surface of the gel-tube.

A 5 mg sample of the granules was flushed into the tube with 20 ml of purified water or HPC solution. The holding ratio of the granules was calculated subtractively by measuring the amount of B.B. in the solution flowing out.

In the experiment for determining the effect of viscosity of the flow solution, 0.1, 0.3, 0.5, 0.65, 0.75, 1.0 and 1.1% HPC solutions were used. The kinematic viscosity of HPC solutions was evaluated at $37 \pm 1°C$ using an Ubbelohde viscometer.

The effect of premagnetization of the granules was determined by allowing 5 mg of the granules to stand within a magnetic field of about 700 G for a predefined period, followed by immediately flushing with 20 ml of 0.65% HPC solution.

*In vivo studies using rabbits*

Male New Zealand White rabbits (approx. 2.5 kg body weight) were used in all experiments. As shown in Fig. 2, a magnetic circuit (Sumitsu & Co.) generating a magnetic field of about 1900 G at point P was used in the experiments. Rabbits were anesthetized with pentobarbital sodium (25 mg/kg, i.v.) after fasting for more than 12 h and fixed supinely at an angle of approx. 45°, as shown in Fig. 3. The area around the pharynx of the rabbit was incised for exposure of the esophagus. A catheter was inserted into the esophagus, confirming that the point of the catheter did not reach the position where the magnets were applied. Granules (5 mg) containing 50% ferrite were administered through the catheter

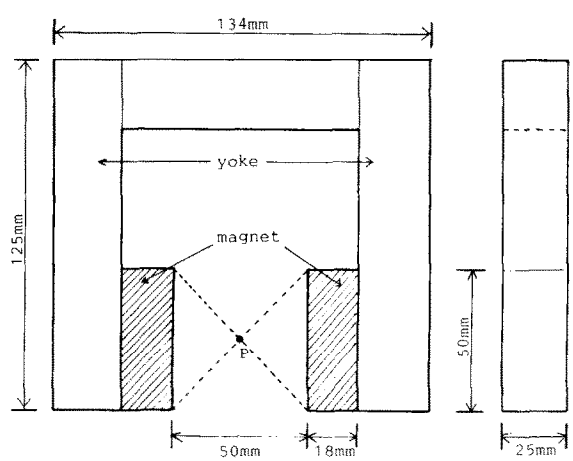


Fig. 2. Illustration of the magnetic circuit used for the in vivo study.

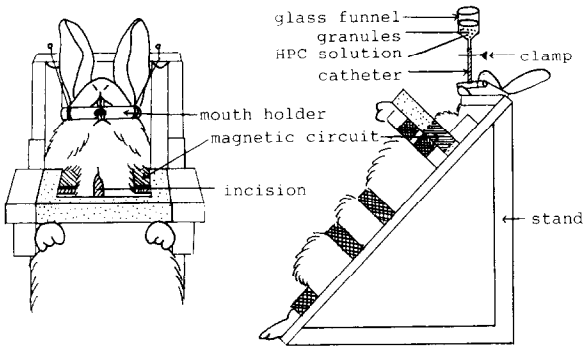


Fig. 3. Administration of granules for the study in vivo.

with 2 ml of 0.65% HPC solution at approx. 1 h after anesthesia when the rabbit was in emergence, except for local anesthesia at an area around the pharynx by lidocaine injection.

At a predefined time after administration, the rabbit was killed immediately and the esophagus excised. The esophageal mucosa was incised and divided into five segments including the part where the magnets were applied, as shown in Fig. 4. B.B. remaining on each segment of mucosa and in the granules remaining on segment No. 2 was then measured. After recovering the granules from the mucosal segment, each segment was cut into pieces with a surgical knife. Each mucosal sample and the granules recovered were separately stirred in 8 ml of ethanol, overnight, and adjusted to 10 ml with ethanol followed by centrifugation at 3000 rpm for 10 min. The amount of B.B. in the supernatant was determined as described above.

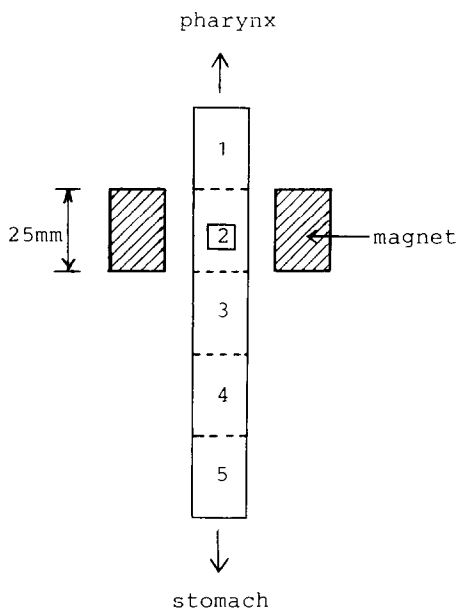


Fig. 4. Division of the esophageal mucosa of rabbit for evaluation of magnetic targeting.

The amount of ferrite in the recovered granules was determined by using *o*-phenanthroline as follows. After release of B.B., granules were dried in vacuo overnight and boiled for 5 min with 25 ml of diluted HCl ($1 \rightarrow 2$) and 5 ml of $H_2O_2$, cooled to room temperature, and adjusted to 100 ml with purified water. Subsequently, 2 ml of hydroxylamine hydrochloride solution (1% w/v) was added to 10 ml of the above solution, 50 ml of purified water was added, and the pH was adjusted to about 4.0 with 30% sodium acetate solution. Next, 5 ml of *o*-phenanthroline solution (0.18% w/v) was added and the volume was adjusted to 100 ml with purified water. After 15 min, the absorbance of the solution was measured at 510 nm, and the $Fe^{2+}$ content was determined, based on the calibration curve for an $Fe^{3+}$ standard solution.

To serve as a control, part of the esophageal mucosa without the administration of granules was washed out with ethanol, the precipitate obtained was dried in vacuo overnight and the $Fe^{3+}$ content was assayed.

## Results and Discussion

*Selection of bioadhesive polymers for the preparation of granules*

Eight kinds of polymers, which were expected to provide bioadhesion on the surface of the target site and sustained drug release, were examined using the B.B. release test with an agar-gel bed. The patterns of B.B. release from each granule are shown in Fig. 5.

The release of B.B. from six kinds of granules was sustained properly, except for those containing HPMC which induced excessively rapid release. As a result, an HPC/CP mixture in the ratio 6 : 4 w/w was chosen as polymer, since both are soluble in ethanol and thus less preparation time is required than with the others. Moreover, the

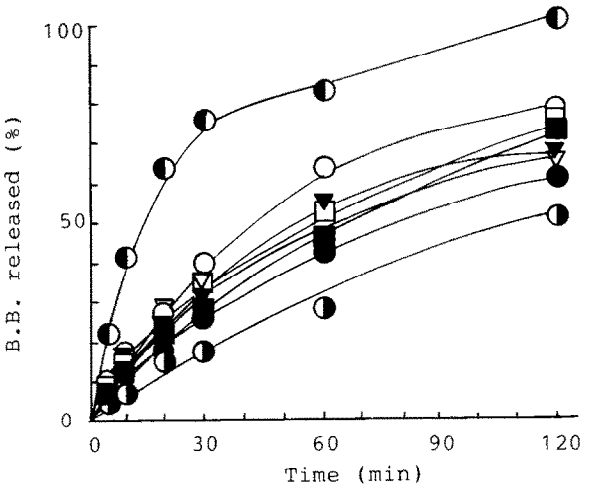


Fig. 5. Release patterns of B.B. from granules obtained by the agar-gel bed method. ◐, HPMC; ○, tamarind gum; □, tragacanth; ■, HPC:CP (6:4); ▼, AA-Na; ▽, PAA-Na; ●, carrageenan; Ⓓ, CMC-Na.

degree of bioadhesion of this mixture has been reported (Machida et al., 1979) and the granules containing the above mixture indicated an appropriate extent of sustained release.

*Effect of viscosity of flow solution, premagnetization of granules, ferrite content and magnetic force on holding tendencies of granules*

It was considered that the ability of the external magnets to hold magnetic granules was governed by the viscosity of the flow solution, granule premagnetization, ferrite content and magnetic field strength. Therefore, the effect of these factors on the holding of magnetic granules was examined.

The effect of viscosity of the flow solution is shown in Fig. 6. A 0.65% w/v HPC solution with a viscosity of 46 cst (centistokes) was found to give the maximum value for the holding ratio of B.B. The result suggests that HPC solutions of viscosity above 46 cst disturb the holding of the granules. Therefore, 0.65% HPC solution of viscosity 46 cst was selected as the flow solution in the following experiments.

The effect of premagnetization is also depicted in Fig. 6. The holding ratio of B.B. was increased by magnetization for 10 s and then reached a plateau. This suggests that premagnetization of granules should be effective at increasing the holding ratio. However, premagnetization induced the agglomeration of granules and, hence, was not employed in the following experiments.

The ferrite content and strength of the magnetic field also affected the holding ability, the holding ratio of B.B. increasing almost directly in proportion to the ferrite content in a field of 700 G, as shown in Fig. 7. For a magnetic field strength of about 1700 G (magnets no. 2 in Fig. 1), the holding ratio of granules containing more than 50% ferrite reached the maximum level.

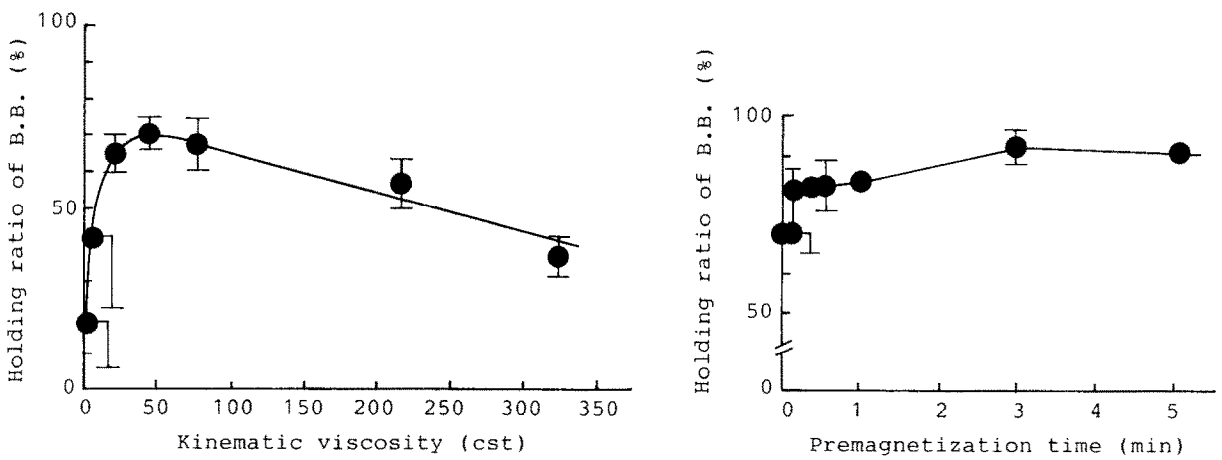


Fig. 6. Relations between kinematic viscosity of flow solution or premagnetization time and holding ratio of B.B. at 700 G. Each value is the mean ± S.E. ($n = 3$).

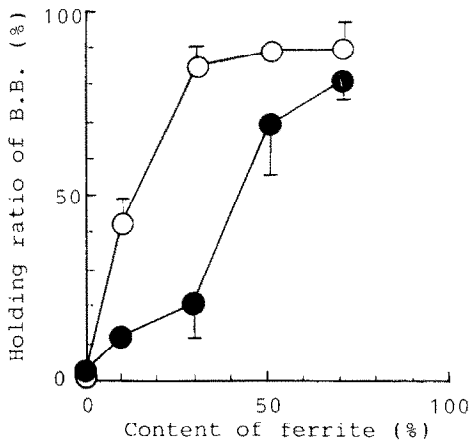


Fig. 7. Relation between ferrite content and holding ratio of B.B. ●, 700 G; ○, 1700 G.

*Evaluation of targeting by magnetic granules in vivo*

The generation of a magnetic field of strength similar to that in the in vitro experiments was a difficult task when using only a pair of permanent magnets due to the width of the target site in vivo. Therefore, a magnetic circuit consisting of magnets with an iron yoke was designed, generating a magnetic field strength of about 1900 G at the esophagus of the rabbits on application at the neck, as shown in Fig. 3.

On comparing the esophagus excised at 2 h after administration of magnetic granules together with application of the magnetic circuit with that at 15 min after administration without the circuit, granules were observed only in the region where the circuit was applied. This demonstrates that the magnetic granules were guided under the influence of the magnetic field to the region where the circuit was applied. The results obtained for other periods of application of the circuit, viz., for 30 min or 1 h after administration, were identical with those for 2 h application.

The distribution of B.B. in each segment of esophagus at a predefined interval after oral administration of magnetic granules is illustrated in Fig. 8. In the absence of the circuit, B.B. was detected in none of the segments of esophageal mucosa. When the circuit was applied for 30 min, 1 h or 2 h after administration, the proportion of B.B. remaining in segment no. 2, where the circuit was applied, was 13.14, 8.15 or 4.27%, respectively. These values are significantly greater compared to those of other mucosal segments without the circuit being applied (Nos 1, 3–5), and are in accord with the results of visual observations as described above.

Ferrite in the granules recovered from segment no. 2 was measured as $Fe^{2+}$, yielding the results shown in Fig. 9. Without the circuit being applied,

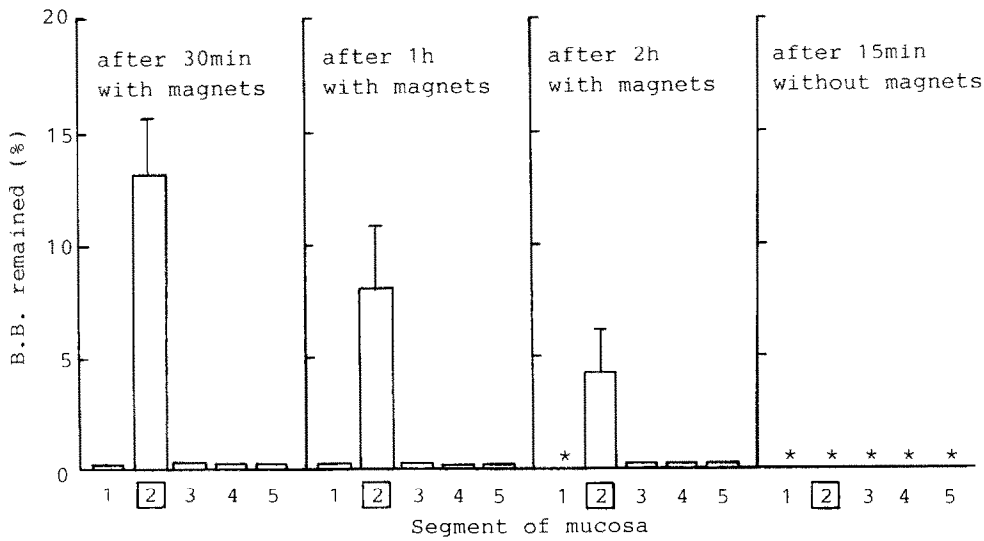


Fig. 8. Effect of magnetic field on proportion of B.B. remaining. Each value is the mean ± S.E. * Not detected.

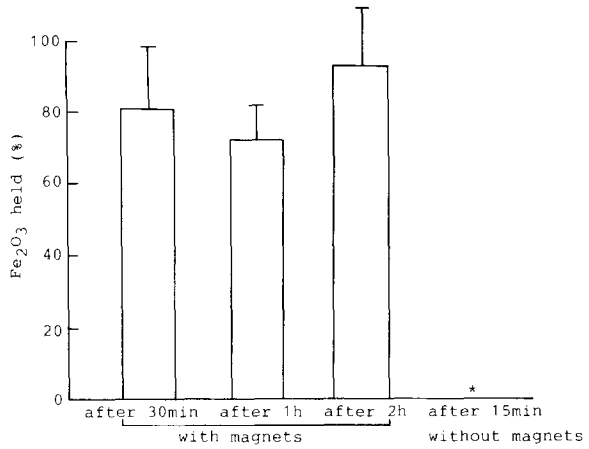


Fig. 9. Effect of magnetic field on holding ratio of $Fe_2O_3$ at the targeting mucosa (segment no. 2). Each value is the mean ± S.E.
* Not detected.

ferrite was undetectable even on segment no. 2. Moreover, there was no significant difference between all three values obtained with application of the magnets for 30 min, 1 h and 2 h. Consequently, it was assumed that the holding situation of the granules remained unaltered for at least 2 h after administration.

*Effects of bioadhesive polymers in granules on targeting in vivo*

Fig. 10 shows esophagi excised at 2 h following administration of magnetic granules with application of the magnetic circuit for the initial 2 min (a) and for 2 h (b). The residual content (%) of B.B. and ferrite is shown in Fig. 11 for both cases. No significant difference was observed between the values for 2 min and 2 h application of the field in both the holding situation (Fig. 10) and the holding ratio of ferrite (Fig. 11).

The granules were considered to adhere to the surface of the mucosa due to the adhesiveness of the polymers combined in the granules even after removal of the circuit and hence, it was demonstrated that 2 min under the influence of the magnetic field after administration was sufficient to fix the granules within this region for 2 h.

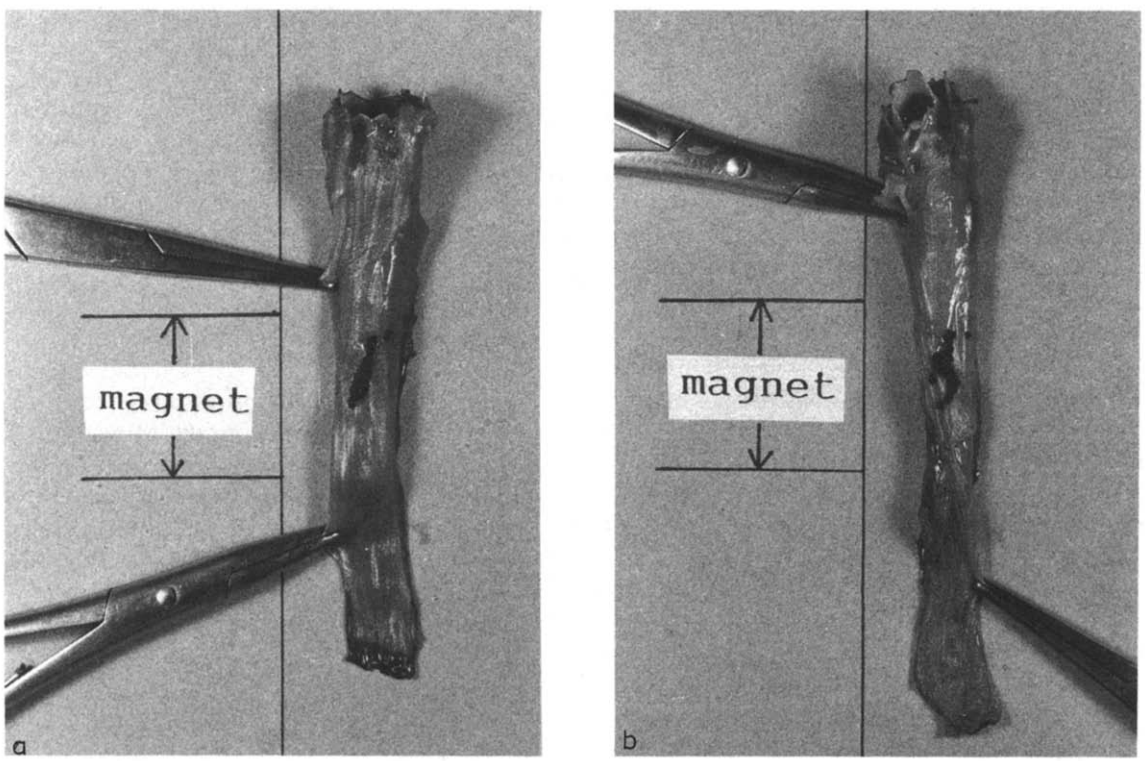


Fig. 10. Esophagi excised at 2 h after administration of the magnetic granules with application of the magnetic circuit for (a) the initial 2 min and (b) 2 h.

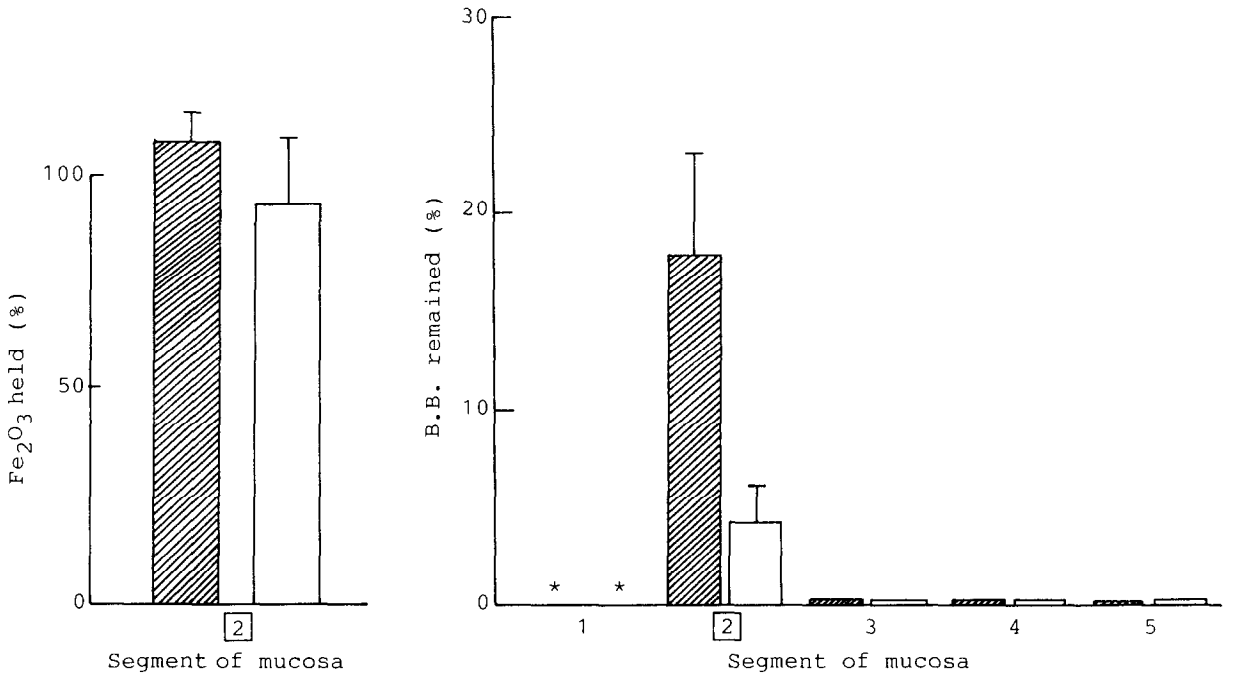


Fig. 11. Effect of application time of the magnetic circuit on holding ratios of $Fe_2O_3$ and B.B. at 2 h after administration. Magnetic circuit was applied for: (hatched bars) the initial 2 min, and (empty bars) 2 h. * Not detected. Each value is the mean ± S.E.

Nevertheless, the proportion of B.B. remaining in the region was significant in the case of a 2 min application of the magnetic circuit (Fig. 11). Siegel et al. (1984) reported a magnetically controlled release system with small magnetic beads in which drug release can be accelerated by application of an oscillating magnetic field via oscillation of the beads by the magnetic force. Therefore, it is considered that the ferrite particles in the granules were attracted by the stronger magnetic field during application of the circuit, so that the release of B.B. from the granules was accelerated. This tendency should be utilized for the control of drug release from the magnetic granules which adhere to mucosa.

## Conclusion

The application of magnetic granules containing bioadhesive polymers as a drug delivery system for anticancer drugs should be advantageous in targeting local chemotherapy for esophageal cancer and other diseases of the alimentary canal. The present system might gain in usefulness from further investigations to devise a more reliable technique to generate a magnetic field at a target and control of drug release.

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