[Chem. Pharm. Bull. 27(1) 93—100 (1979)]

UDC 615.277.3.014.2.036 : 615.453.011.4.014

# Preparation and Phase II Clinical Examination of Topical Dosage Form for Treatment of *Carcinoma Colli* Containing Bleomycin with Hydroxypropyl Cellulose[1,2]

Yoshiharu Machida,[3a] Hiroshi Masuda, Norimasa Fujiyama, Susumu Ito,[3b] Masanori Iwata, and Tsuneji Nagai[3a]

*Hoshi Institute of Pharmaceutical Sciences[3a] and Hospital of Yokohama City University, School of Medicine[3b]*

(Received July 17, 1978)

A new topical disk-like dosage form for *carcinoma colli* was prepared by direct compression of the mixture of bleomycin hydrochloride (BLM) and combination of hydroxypropyl cellulose (HPC) and other water soluble polymer. After *in vitro* examinations regarding the water adsorption, the release and dissolution of drug and other properties, the preparations were examined clinically by applying to the voluntary patients.

A combination of HPC and Carbopol 934 (CP) seemed preferable as the vehicles, and the amount of BLM released from the preparation increased remarkably with an increase in concentration of HPC. In contrast, the water absorbing property increased with an increase of CP.

The preparation containing 30 mg of BLM, for which the preparative formula was designed according to the *in vitro* experiment, was administered to the voluntary patients of *carcinoma colli* of stage 0 to Ib. The preparation taken out of patient indicated the continuous release of BLM for longer than 24 hr. In the three of nine patients, any cancerous focus was not found after the local therapy using 90 to 195 mg of BLM in total, and remnant foci found in the other six patients were very few. Moreover, the normal mucosa was not affected by BLM, contrary to the case of usual suppositories reported. The higher per cent of cure might be expected if the therapy could be continued for the longer period.

**Keywords**——clinical examination; topical dosage form; *carcinoma colli*; hydroxypropyl cellulose; bleomycin; releasing property; dissolution test; local therapy; sustained release

Uterine cancers are known to occupy about 25% of all female malignant tumors in Japan among which *carcinoma colli* occupies 95%.[4] The *carcinoma colli* is generally treated by surgical operation, *i. e.*, removing the whole uterus accompanying subsidiarily a radiation therapy or a drug treatment. The surgical operation brings about a cure with a high accuracy, especially in early stage of *carcinoma colli*. However, the extirpation of the whole uterus often gives not only various bad effects on the body, but also it offers a serious problem particularly for young patients who want to become pregnant. Moreover, such the subsidiary radiation therapy or drug treatment after the operation is not a faultless method because of its adverse actions. The drug treatment mentioned above is concerned with the systemic administration of drug. Therefore, if some dosage form can be provided to make the drug reach the cancer cells with a high selectivity, a drug treatment for *carcinoma colli* may go beyond the subsidiary one. On the early stage of *carcinoma colli* such as stage 0 or Ia, cancer

1) This paper forms Part XIII of "Pharmaceutical Interactions in Dosage Form and Processing." Preceding paper, Part XII: Y. Takahashi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **26**, 3836 (1978).

2) A part of this work was presented at the 97th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1977.

3) Location: *a*) *Ebara-2-4-41, Shinagawa-ku, Tokyo, 142, Japan*; *b*) *Urafunecho-3-46, Minami-ku, Yokohama-shi, 232, Japan.*

4) T. Suzuki, "Shikyu-gan Shindan no Tebiki," Kinbara Shuppan Co. Ltd., Tokyo, 1973.

cells stay at or near epithelium, and thus it seemed possible to bring about a cure by topical application of antitumor agent such as bleomycin (BLM). Suppositories for cervical canal containing BLM have been prepared and applicated to the patients of various stage of *carcinoma colli* offering some good effectiveness.[5] However, there has been observed such a disadvantage that the drug release is so rapid as to cause an inflammation of healthy vaginal mucosa.[5]

The present study was attempted to design a new topical dosage form for *carcinoma colli* wearing the following three suitable characteristics: the release of drug, the swelling of the preparation without dispersion and the adhesiveness to the disease part. This idea came from the authors' previous works concerning a pharmaceutical application of hydroxypropyl cellulose (HPC).[6,7] BLM and a combination of HPC and other water soluble polymer were used as the active ingredient and the vehicles, respectively. Rosaniline hydrochloride and brilliant blue FCF were used for preliminary *in vitro* examinations instead of BLM because they were easily soluble in water, visible and spectrophotometrically determinable. After *in vitro* examinations regarding the water absorption, the release and dissolution of drug and other properties, the preparations were examined clinically by applying to the voluntary patients in various stages of *carcinoma colli*. As a result, the release of drugs was sustained over 23 hr, *i. e.*, the cancer cell cycle. The clinical result suggested that the present dosage form might afford a promising means for the treatment of *carcinoma colli*.

## Experimental

**Materials**——Hydroxypropyl cellulose-H (HPC),[8] Carbopol 934 (CP),[9] agar powder J.P. IX and polyethylene oxide (PEO)[10] used were commercially supplied. Bleomycin hydrochloride J.P. IX (BLM), freeze dried product for injection, was supplied from Nihon Kayaku Co. Ltd. Rosaniline hydrochloride (RAH) and brilliant blue FCF (BB) used were of the reagent grade.

**Preparation of Dosage Form**——Flat faced disks of 300 mg, 13 mm diameter and about 2.0 mm thickness were made by compressing the powder mixture directly under 200 kg/cm$^2$ for 30 sec using a Shimadzu evacuable die and hydraulic press for KBr tablet for infrared spectroscopy.

**Measurement of the Rate of Release of RAH, BB or BLM from Disk**——Agar plate was chosen as the simple model of mucosa because it can keep the adequate amount of water similar to secreting fluid which is required for swelling of preparation and release of drug.

The releasing rate of drug was measured by the following three methods. Many trial such as the methods described here may be required prior to the establishment of evaluation method for the new dosage form.

I) Method 1: This was to evaluate roughly the releasing property and change of shape by watching the colored part by the pigment. Each of the disks shown in Table I was put on the center of 1% agar

Table I. The Formula of the Disks examined by Method 1

| Disks | HPC | CP | PEO | HPC:CP 5:1 | HPC:CP 3:1 | HPC:CP 1:1 |
|---|---|---|---|---|---|---|
| RAH (mg)[a] | 15 | 15 | 15 | 15 | 15 | 15 |
| HPC (mg)[a] | 285 | — | — | 237.5 | 213.75 | 142.5 |
| CP (mg)[a] | — | 285 | — | 47.5 | 71.25 | 142.5 |
| PEO (mg)[a] | — | — | 285 | — | — | — |

a) Amount contained in 1 disk.

5) H. Masuda, Y. Sumiyoshi, and Y. Shiojima, at the 14th Congress of Japan Society for Cancer Therapy, Sendai, Oct. 1976.
6) Y. Machida and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **22**, 2346 (1974).
7) Y. Machida and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **26**, 1652 (1978).
8) Product of Nippon Soda Co. Ltd., the viscosity of 2% aqueous solution was 2744 cps at 20° when determined by a Tokyo Keiki BL type viscometer.
9) Carboxypolymethylene, product of B.F. Goodrich Chemical Co.
10) Product of Nippon Soda Co. Ltd.

plate in a petri dish of 90 mm diameter. The petri dish was kept in incubator (37°±1°) and the area colored by RAH released into agar plate was measured by a slide caliper at 3, 6, 12, 18, 24 and 48 hr. The change of shape of disk following the absorption of water was also observed simultaneously.

II) Method 2: In this method, releasing rate was evaluated from the amount of pigment remained in disk. BB was used in this method because the blue pigment was convenient for spectrophotometrical determination. The disk containing 10 mg of BB was put on an agar plate in the same way as Method 1. After 6, 24, 48 or 72 hr, the disk was transfered into 50 ml of purified water and allowed to stand for 30 hr at 37°±1° in a incubator. The concentration of BB in water, which corresponded to the amount of pigment remained in the disk in the above experiment, was determined spectrophotometrically at 630 nm using a Hitachi 124 spectrophotometer.

III) Method 3: This was applied for the preparation containing active ingredient and BLM released was directly determined by spectrophotometry after extraction from agar plate. The disk containing 30 mg of BLM was put on agar plate and picked up after 6 or 24 hr. The surface of agar plate was wiped gently by wet gauze, and then the agar layer was homogenized in 50 ml of purified water for 5 min by a Nihon Seiki homogenizer. The homogenized mixture was centrifuged in a glass stoppered centrifuge tube for 10 min at 3000 rpm. The concentration of BLM in the supernatant solution, which corresponded to the amount released from disk, was determined in reference to the calibration curve at 290 nm by a Hitachi 124 spectrophotometer.

**Measurement of Dissolution Rate**——The disk containing 10 mg of BB was examined by a Toyama Sangyo TR-5S3 dissolution tester[11] in 900 ml of purified water and at 200 rpm revolution speed of basket. BB was convenient for spectrophotometry because of its blue color and moreover it was easily washed off from testing vessels. Ten milliliter of the dissolution medium in testing vessel was pipetted out at adequate time intervals and the concentration of BB was determined in reference to the calibration curve at 630 nm. The resultant want of dissolution medium was conpensated after each sampling by adding the same volume of purified water prewarmed in the same temperature.

**Measurement of Water Absorbing Property of Disk**——The amount of water absorbed was determined by weighing the disk before and after standing on the agar plate for 6, 24, 48 or 72 hr.

**Clinical Application**——The disk containing 30 mg of BLM held in HPC and CP (1: 1 to 1: 2) was subjected to the clinical examination on nine voluntary patients of various stages of *carcinoma colli*, prior to the surgical operation. One disk a day was set on *portio vaginalis* up to 90 mg to 195 mg[12] in total amount administered. The lesion was observed occasionally by colposcope through all the period of treatment. After the extirpation of uterus, preparations of histological section were made of extirpated *cervix uteri* by a conventional method, and then examined microscopically whether the cancer cell remained or not.

## Results and Discussion

### Release of Pigment from Disk determined by Method 1 and Evaluation of Vehicles

A preliminary rough evaluation of vehicles was carried out by examining the release of pigment from the disk and the change of shape following the absorption of water. Figure 1 shows the increasing area colored by the pigment released from disk with the elapse of time. In the case of disk consisting of HPC and CP, the release of pigment increased with an increase in concentration of HPC, getting to the greatest in the disk of HPC alone. In contrast, the disk of CP alone gave a low releasing rate. Therefore, HPC was considered to improve the releasing property in the case of disk consisting of HPC and CP. The difference in releasing property between the disks of HPC and CP may come from that in water solubility, *i. e.*, higher for HPC than for CP.

The disk of PEO alone also indicated a good releasing property similar to HPC. However, both disks of PEO and HPC were dissolved completely after about 18 hr and 24 hr, respectively. On the other hand, the disk of CP changed only to a swollen shape with a high elasticity. Therefore, a combination of HPC and CP seemed preferable as the vehicles, and thus further experiments were carried out using the disks of mixture of HPC and CP.

### Release of Pigment from Disk determined by Method 2 and Water Absorbing Property

The data obtained by Method 1 gave a rough knowledge about the pigment releasing property of disk. Therefore, a precise measurement of the amount of pigment remained in

11) The same type as that in U.S.P. XIX.

12) 195 mg means that a halved disk and 30 mg disk were subjected once and six times, respectively.

disk after standing on the agar plate was carried out by Method 2 and also the water absorbing property was examined simultaneously. Figure 2 shows that the releasing rate increased with an increase in concentration of HPC, as was similar to the result obtained by Method 1, and this tendency was remarkable in the experiment for comparatively short standing periods, *i. e.*, 6 hr or 24 hr. A low increase of release with an increase in concentration of HPC observed after 72 hr or 48 hr was considered to have relation to the evaporation of water

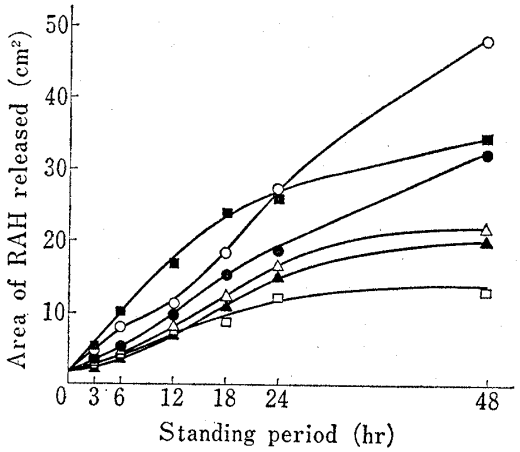


Fig. 1. Pigment Releasing Profiles of Various Disks obtained by Method 1

—○—: HPC, —■—: PEO, —□—: CP, —●—: HPC:CP=5:1, —△—: HPC:CP=3:1, —▲—: HPC:CP=1:1.
Each symbol represents the mean of 3 determinations.

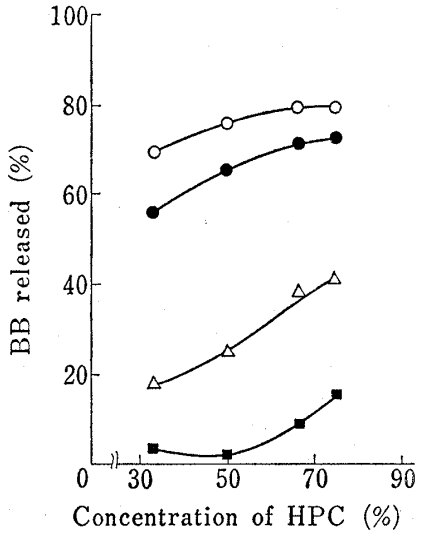


Fig. 2. Relation between Pigment Releasing Property of the Disk determined by Method 2 and Concentration of HPC

—■—: 6 hr, —△—: 24 hr, —●—: 48 hr, —○—: 72 hr.
Each symbol represents the mean of 3 determinations.

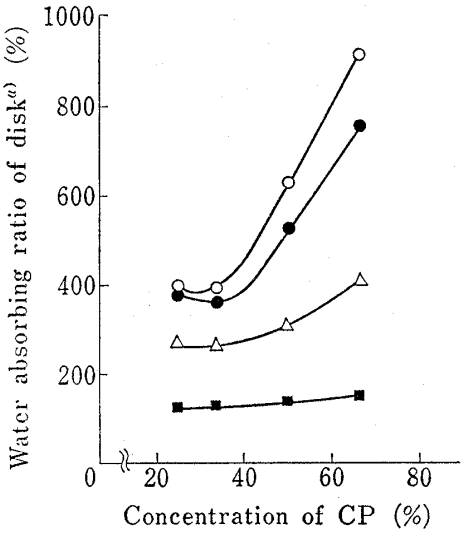


Fig. 3. Relation between Water Absorbing Property of the Disk and Concentration of CP

—■—: 6 hr, —△—: 24 hr, —●—: 48 hr, —○—: 72 hr.
Each symbol represents the mean of 3 determinations.
*a*) (weight of water absorbed/weight of disk before swelling) × 100.

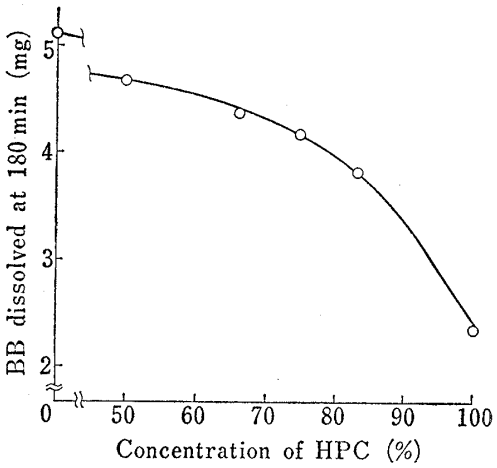


Fig. 4. Relation between BB dissolved at 180 min and Concentration of HPC

Each symbol represents the mean of 3 determinations.

from the agar plate. As shown in Fig. 3, contrary to the releasing property mentioned above, the water absorbing property increased remarkably with an increase of CP, and this tendency was clear in the experiment for long standing periods, suggesting that there was required some induction period for the water absorption by CP. Therefore, it seemed reasonable that the absorption of water by disk caused the loss of water from the agar plate resulting in the retardation of release of pigment after long standing periods. The loss of water from the agar plate might take place not only on the interface between the disk but also more or less from all the surface of the agar plate. However, assuming the disk is set in the body, such the loss of water may not take place because the secreting fluid is continuously supplied to the disk, and thus the release may be faster than *in vitro* cases. This kind of consideration should be important in designing a formula of the preparation.

**Dissolution of Pigment from Disk**

The dissolution test usually gives various informations regarding the drug releasing property of solid preparations. In this study, as shown in Fig. 4, the dissolution rate of BB decreased with an increase in concentration of HPC, as was quite different from the relation between releasing property and concentration of HPC mentioned already. Such the opposite results might come from the difference in the situation and quantity of water around the disk between both the experimental methods. In the experiments using agar plate, HPC might dissolve gradually in the water included in swollen agar plate, making a pathway for pigment. On the other hand, in the experiment using a U. S. P. type dissolution tester, a large volume of water surrounding the disk might promote the formation of a gel-like layer of HPC on the surface of disk which suppresses the quick penetration of water, resulting in a retardation of the dissolution of pigment, as was reported regarding the disintegration of the tablet containing a large volume of HPC.[6]

**Release of Bleomycin Hydrochloride from Disk determined by Method 3**

As shown in Fig. 5, the amount of BLM released increased remarkably with an increase in concentration of HPC as was similar to the result shown in Fig. 2.

This result simply showed that the disk containing comparatively high concentration of HPC might be preferable to make the dosage form holding a good releasing property. However, the present *in vitro* experimental conditions were not the same as those on the actual disease part. The difference in the amount of secreting fluid, form of the disease part and other conditions of each individuals seemed to give effect on the releasing property

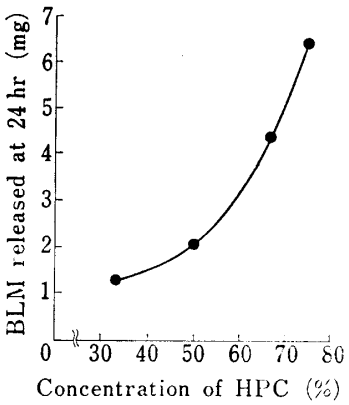


Fig. 5. Relation between Release of BLM from the Disk determined by Method 3 and Concentration of HPC

Each symbol represents the mean of 3 determinations.

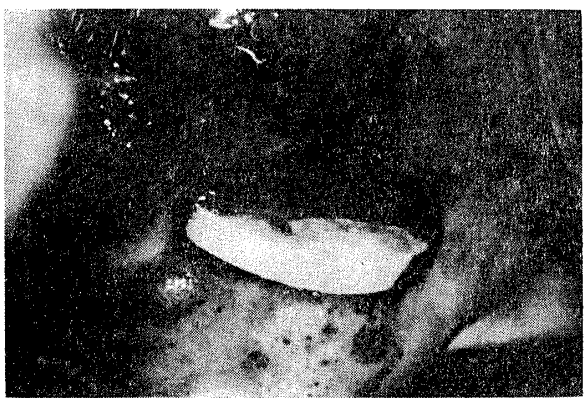

Fig. 6. Disk inserted into External Os of the Uterus of Subject T.S.

or the durability of the disk *in vivo*. Further improvement should be required to gain a more reliable and suitable releasing property of disk.

**Clinical Application and Evaluation of Effectiveness of Preparation**

The result obtained from *in vitro* preliminary experiment suggested that the disks containing 33, 50, 69 and 75% HPC could be subjected to a clinical examination. In this connection, CP has been used generally for cosmetics, ointment base *etc.*, and its toxicity had been reported as negligible.[13] Moreover, Tamura *et al.* reported that the tissue after extraction of tooth was not affected by the contact of CP during 24 hr in an experiment using rabbit.[14] In this study, in order to confirm that CP can be used clinically with a high safety, the disk containing no BLM (50% HPC) was examined by setting on *portio vaginalis* of voluntary patients for 24 hr. As a result, it was confirmed that the disk did not give any injurious effect to mucosa, remaining on the set place as in swollen state.

Then, the disk containing 30 mg of BLM held in HPC and CP (1: 1 to 1: 2) was administered to voluntary patients of *carcinoma colli* of stage 0 to Ib as mentioned previously. Figure 6 shows *portio vaginalis* of subject T. S. diagnosed as stage Ib of *carcinoma colli*, where the disk was inserted into the external os of uterus. The disk usually can be used by sticking to *portio vaginalis*. This disk shown in Fig. 6 was inserted into cervical canal because the lesion existed there in the case of this subject. Figure 7 shows the disk taken out of patient after 24 hr enlarged by swelling to about two times in diameter compared with the initial size. Many foams were observed in the swollen gel-like part of the disk and moreover the opaque part where the secreting fluid did not penetrate into was also observed in the center of the aggregate. It was suggested, therefore, that the continuous release of BLM might take place for longer than 24 hr. According to the classification of antitumor agents by Shimoyama *et al.*,[15] BLM belongs to the type Ib, whose cell killing activity depends on the concentration of drug and the period of contact to cancer cells. Therefore, a moderate release rate of BLM from the present dosage form may enhance the cell killing action of BLM. In other words, this dosage form could improve the usefulness of BLM.

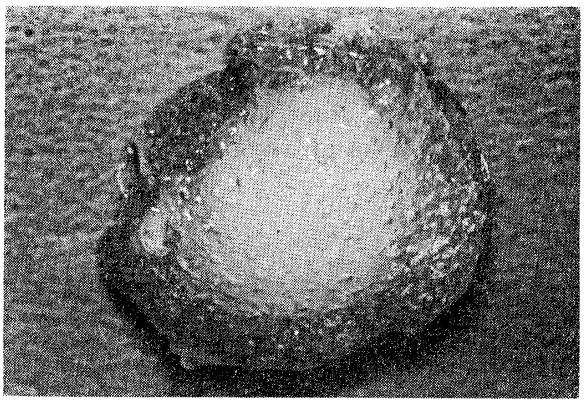

Fig. 7. Swollen Disk taken out from Subject T. S. after 24 hr

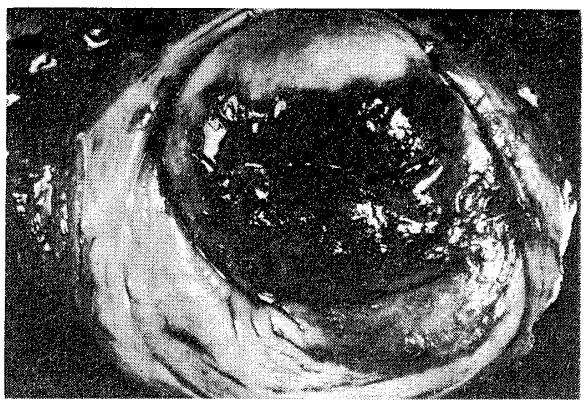

Fig. 8. *Portio Vaginalis* and a Part of Vagina removed from Subject T. S. after Local Therapy

Figure 8 shows *portio vaginalis* and a part of vagina removed from patient T. S. after the local therapy using 150 mg of BLM in total. From colposcopic observation, it was observed that the lesion was changed to a rough surface due to the necrosis caused by the

13) B.F. Goodrich Chemical Co., Service Bulletin GC-36, Revised, 1970.
14) T. Tamura, A. Fujii, and S. Kobayashi, *The Clinical Report*, **11**, 1352 (1977).
15) *a*) M. Shimoyama, *Saishin Igaku*, **28**, 850 (1973); *b*) M. Shimoyama and K. Kimura, *Saishin Igaku*, **28**, 1024 (1973); *c*) M. Shimoyama, *Gan to Kagakuryoho*, **3**, 1103 (1976).

attack of BLM, but the normal mucosa was not affected by BLM. In this connection, usual suppositories containing BLM in Imuhausen base[5] are known to cause necrosis even on the normal mucosa. Schematic comparisons in focus of *cervix uteri* before and after surgical operation are shown in Fig. 9. *Portio vaginalis* is symbolized as circle and the form of the external os of uterus is in it. Moreover, the oblique lined part in the left circle indicates the lesion presumed from colposcopic observation, and the point in the right circle indicates the position and number of remnant of cancerous focus observed in histological section of the uterus removed after the local therapy.

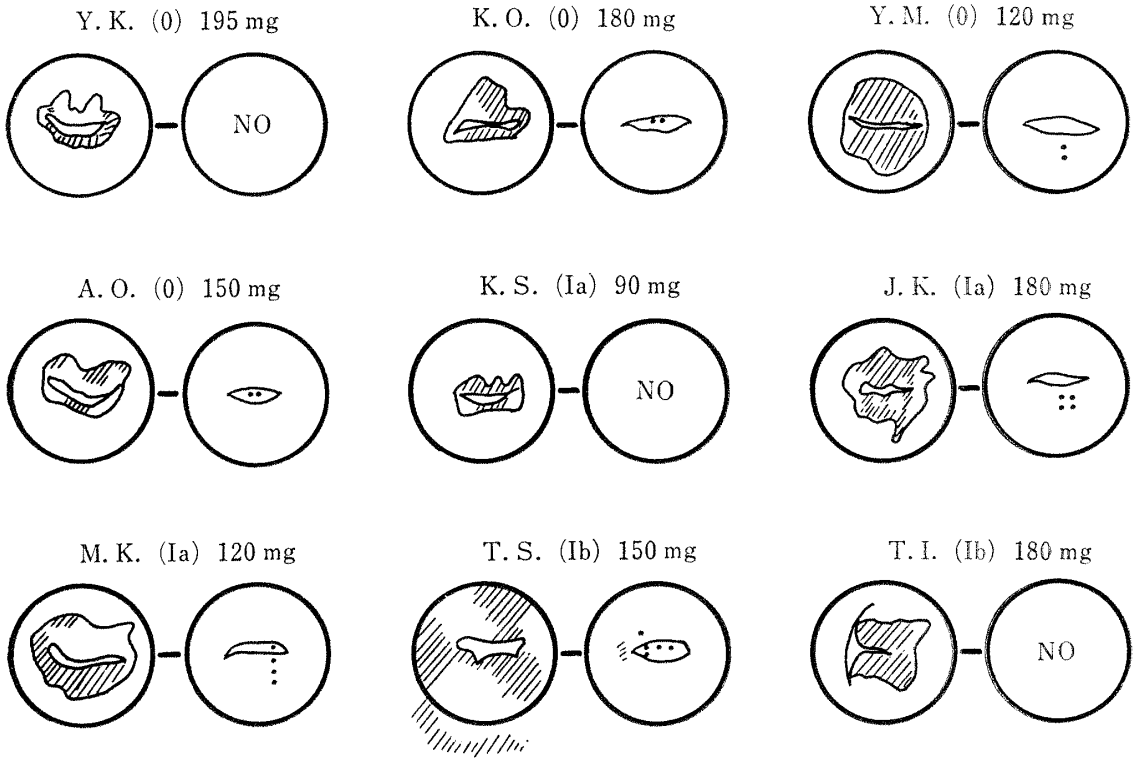


Fig. 9

In the cases of patients Y. K. (stage 0), K. S. (stage Ia) and T. I. (stage Ib), any cancerous focus was not found after the local therapy using 195 mg, 90 mg and 180 mg of BLM, respectively. The cancerous focus did not disappear completely in the six patients of the nine until the operation, but the number of remnant focus was very few. Therefore, it can be said that the local therapy using the presented dosage form containing BLM may bring about a considerable cure. Papanicolaou smear of vagina indicated the above mentioned cases *i. e.*, patients J. K., K. O., M. K. and A. O., changed after the treatment from class IV to III, class V to III, class IV to II and class IV to II, respectively.[16]

The percentage of perfect disappearance of cancerous focus in above mentioned cases was 33%. This was not so high because the term of administration was three weeks at the longest, *i. e.*, two times a week and six times in all. Therefore, the higher per cent of cure might be expected if the therapy could be continued for the longer period. Recently, the technique and system of an early detection of uterine cancer has been developed, *e. g.*, by a mass screening. And thus the local therapy using the presented topical dosage form may

16) Papanicolaou's classification: class I: absence of atypical or abnormal cells; class II: atypical cytology but no evidence of malignancy; class III: cytology suggestive of, but not conclusive for, malignancy; class IV: cytology strongly suggestive of malignancy; class V: cytology conclusive for malignancy.

afford a useful means for the treatment of the patients in early stage of disease who dislike the surgical operation, or the patients of cancer progressed unfavorably for whom the operation and the radiation therapy can not be effective.

Further investigations should be made to find more suitable vehicles, active ingredient and shape of dosage form[17] for an improvement of therapeutic effect and safety.

**Acknowledgement** The authors are very grateful to Mr. Hideo Akiyama and Miss Kayoko Ikeshita for their assistance in the experimental work.

17) *e.g.*, Y. Machida, H. Masuda, N. Fujiyama, M. Iwata, and T. Nagai, Presentation No. 5E10-4, at the 98th Annual Meeting of Pharmaceutical Society of Japan, Okayama, April 1978.