Table IV. Conjugation of Fluorescein in the Urine of Wistar Rats (Male, 250 g) administered 10 mg/kg of Fluorescein

	n	FG/TF±S.D. (%)
Normal rats	6	11.2±3.8
Acute CCl_4 injury	6	4.4±6.3
Acute D-(+)-galactosamine HCl injury	3	5.8±2.5

the first 2 hours in the urine of human subjects orally administered 5 mg of F. Therefore, F may be considered as a possible substrate for testing the liver conjugation function. This possibility was tested by the present analytical method as follows.

Three doses of 0.1, 1.0 and 10 mg per kg body weight of F were separately administered to normal and acutely liver-damaged rats. Although the doses of 0.1 and 1.0 mg/kg did not reflect the conjugation function, the conjugation of F decreased when F was administered at a dose of 10 mg/kg (Table IV). This result shows that a large dose of F may reflect the liver conjugation function.

[Chem. Pharm. Bull. 28(9)2816—2819(1980)]

Sustained Release of Drugs from Ethylcellulose Microcapsules Containing Drugs dispersed in Matrices

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(Received March 27, 1980)

The use of ethylcellulose microcapsules possessing a reservoir system to achieve sustained release of sulfamethizole or 5-fluorouracil was examined *in vitro*. As a first step, the drugs were dispersed in cellulose acetate matrices, then the matrices were microencapsulated by means of coacervation-phase separation of ethylcellulose from diethyl ether solution by nonsolvent addition.

The surfaces of microcapsules were rounded, whereas the milled matrices were sharp-edged. In microcapsules of both drugs, longer release half-lives were obtained than with the corresponding matrices.

Keywords——microcapsule; matrix; sulfamethizole; 5-fluorouracil; coacervation-phase separation; sustained release; photomicrograph

Many attempts have been made to achieve sustained release of drugs.[2,3)] Drugs microencapsulated by poorly water-soluble polymers may be employed for this purpose. The authors have demonstrated the usefulness of enteric coated microcapsules in producing sustained release of an antibacterial agent, sulfamethizole, from microcapsules coated with carboxymethylethylcellulose.[4)] We have also studied ethylcellulose microcapsules containing chemo-

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3) R.W. Baker and H.K. Lonsdale in "Controlled Release of Biologically Active Agents," ed. by A.C. Tanquary and R.E. Lacey, Plenum Press, New York, 1974, pp. 15—71.
4) M. Nakano, M. Ito, K. Juni, H. Sekikawa, and T. Arita, *Int. J. Pharm.*, **4**, 291 (1980).

therapeutic agents as sustained release preparations for antibacterial and anticancer agents.[5]

For the control of some diseases, longer release half-lives of drugs will be required.[6] In our microcapsules prepared by the phase separation method, the drugs were released with half-lives of only 1—2 hr,[5] so methods of obtaining microcapsules with longer half -lives were explored.

In an attempt to examine the applicability of hydrogels for sustained release preparations, the depot system (in which the core portion contains a drug dispersed in a matrix and the peripheral portion contains no drug) has been shown to be very effective in obtaining sustained release of drugs.[7] It may be possible to utilize the same approach in microcapsules.

In this work, microencapsulation of drug-containing matrices in cellulose acetate was examined, and the release patterns of drugs from the resulting microcapsules were compared with those from the matrices.

Experimental

Materials——Ethylcellulose (100 cps) with an ethoxy content of about 49% (lot AIK4978) was purchased from Wako Pure Chemical Ind., Osaka. Cellulose acetate with a combined acetic acid content of 55.1% and a degree of polymerization of 180 was generously supplied by Daicel Chemical Ind., Tokyo. Sulfamethizole of JP IX grade (lot XC14GG) was purchased from Eisai Co., Tokyo, and 5-fluorouracil (lot 27098) was generously supplied by Kyowa Hakko Kogyo Co., Tokyo. All other chemicals were of reagent grade.

Preparation of Matrices——Cellulose acetate and drug powder (3 : 1) were completely dissolved in acetone. A sheet consisting of the drug dispersed in the polymer matrix was obtained by removing the acetone by evaporation at room temperature. The sheet was dried *in vacuo* for 24 hr at ambient temperature. The sheet was then milled in a homoblender (Sakuma Seisakusho, Tokyo) at 18000 rpm and the resulting particles were sieved to collect the 177—297 μm size fraction.

Preparation of Microcapsules——Based on toxicological considerations[8] diethyl ether was chosen as a solvent for ethylcellulose and *n*-heptane as a phase separation agent. The matrices (1 g) were suspended in 5% ethylcellulose solution (40 ml) in diethyl ether and the suspension was stirred for 1—2 hr. *n*-Pentane was added dropwise to the suspension at a rate of 0.5—0.8 ml/min until the phase separation was completed. The microcapsules thus obtained were washed twice with *n*-pentane by mixing and decantation and then collected on a filter paper. The microcapsules were dried *in vacuo* for 24—48 hr at ambient temperature.

Observation of Matrices and Microcapsules by Scanning Electron Microscopy——The dried matrices and the microcapsules were observed with a scanning electron microscope (model S-430, Hitachi Manufacturing Co., Tokyo) to examine their shapes and surface characteristics.

Release Studies——Release profiles of the drug from the matrices and the microcapsules in isotonic tris-(hydroxymethyl)aminomethane buffer solution at pH 7.4 were obtained by a modification of the beaker method reported previously.[4,5] Since one of the objectives in the present study was the development of sustained release microcapsules for transcatheter arterial embolization therapy,[9] release studies were carried out in a medium at neutral pH. The drug concentrations were analyzed spectrophotometrically; sulfamethizole at 262 nm and 5-fluorouracil at 266 nm.

Results and Discussion

Characteristics and Contents of Microcapsules

Scanning electron microscopic observation revealed that the shapes of the microcapsules were indeterminate and rounded at the edges, whereas milled matrices were sharp-edged (Fig. 1). The surfaces of the microcapsules, however, were not very smooth, possibly because ethylcellulose tended to form coacervate droplets on the surfaces of the matrices, resulting in rather tough surfaces in the absence of phase separation-inducing agents.[10] The average

5) M. Ito, M. Nakano, K. Juni, and H. Sekikawa, *Chem. Pharm. Bull.*, **24**, 105 (1980).
6) R.A. Miller, J.M. Brady, and D.E. Cutright, *J. Biomed. Mater. Res.*, **11**, 711 (1977).
7) M. Nakano, K. Takikawa, and T. Arita, *J. Biomed. Mater. Res.*, **13**, 811 (1979).
8) H.E. Christensen, "Toxic Substances Annual List-1971," U.S. Department of Health, Education, and Welfare, Rockville, Md., 1971.
9) T. Kato and R. Nemoto, *Proc. Japan Acad.*, **54**, Ser. B, 413 (1978).
10) I. Jalsenjak, C.F. Nicolaidou, and J.R. Nixon, *J. Pharm. Pharmacol.*, **28**, 912 (1976); P.B. Deasy, M.R. Brophy, B. Ecanow, and M.M. Joy, *J. Pharm. Pharmacol.*, **32**, 15 (1979).

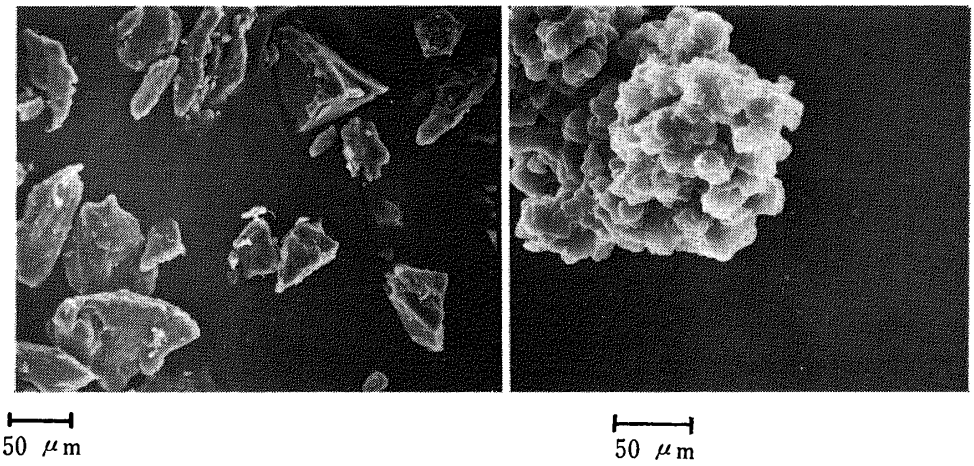

Fig. 1. Scanning Electron Photomicrographs of the Matrices (Left) milled with a Homoblender and the Microcapsules (Right)

diameter of the microcapsules was 297 μm as measured in photomicrographs. Since cellulose acetate, sulfamethizole, and 5-fluorouracil were almost completely insoluble in diethyl ether during the microencapsulation process, the recovery of the drugs in the microcapsules was almost quantitative. The drug contents in the microcapsules were 9% in both cases.

Release Patterns

In Fig. 2, the release patterns of sulfamethizole from the matrices and the microcapsules are compared with the dissolution patterns of the drug powder. Although the drug powder dissolved completely within 10 min, sustained release was obtained from the matrices and the microcapsules. Even in the matrices, a release half-life of about 1 hr was observed. The release of the drug from the microcapsules was further sustained, with a release half-life of about 5 hr. Thus, microcapsules possessing a reservoir system might be useful as a sustained release preparation for antibacterial agents.

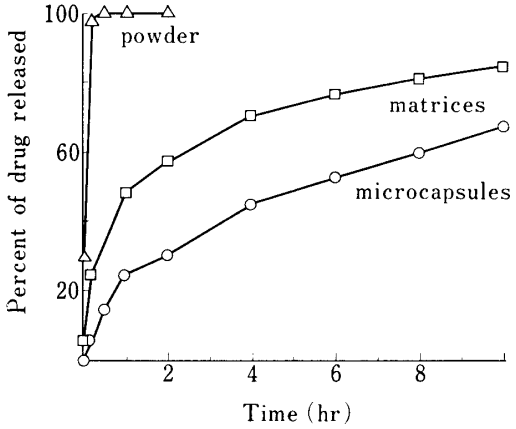

Fig. 2. The Release Patterns of Sulfamethizole from the Matrices (□) and the Microcapsules (○) and the Dissolution Pattern of the Drug Powder (△)

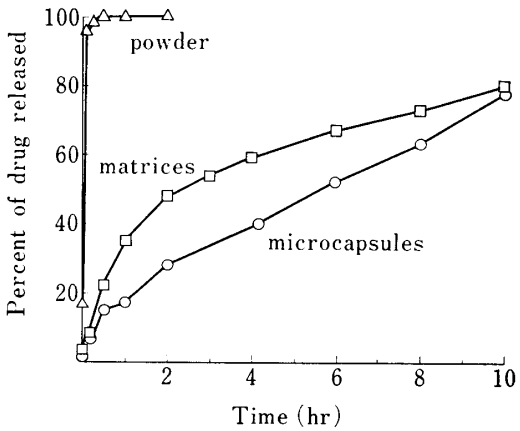

Fig. 3. The Release Patterns of 5-Fluorouracil from the Matrices (□) and the Microcapsules (○) and the Dissolution Pattern of the Drug Powder (△)

The release patterns of 5-fluorouracil from the matrices and the microcapsules are compared with the dissolution pattern of the drug powder in Fig. 3. The release half-life of 2 hr observed in the matrices was extended to 6 hr in the microcapsules. Thus, the sustained

release of the anticancer drug obtained in the present study may be applicable in transcatheter arterial embolization therapy[9] of cancers in kidney and liver.

Acknowledgement The authors are grateful to Daicel Chemical Ind., and Kyowa Hakko Kogyo Co. for generous supplies of cellulose acetate and 5-fluorouracil, respectively.

[Chem. Pharm. Bull. 28(9)2819—2824(1980)]

An Improved Procedure for the Synthesis of 1-Alkyl-5-(alkylamino)-imidazole-4-carboxamides, Synthetic Intermediates for 3,9-Dialkylpurine Derivatives[1]

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Heating N,N-diethyl-3,9-dialkyladeninium halides (Ig—j) in aqueous sodium hydroxide gave 1-alkyl-5-(alkylamino)imidazole-4-carboxamides (IV) together with minor amounts of 1-alkyl-5-(alkylamino)imidazole-4-carbonitriles (III), which were converted into IV on further heating. N,N-Dimethyl-3,9-dialkyladeninium halides (Ia—d) underwent hydrolysis more rapidly to provide IV selectively in 90—94% yields.

Keywords——imidazolecarboxamides; imidazolecarbonitriles; cleavage of purine ring; N,N,3,9-tetraalkyladeninium halides; hydrolysis of amidines; base-catalyzed elimination; dehydration

1-Methyl-5-(methylamino)imidazole-4-carboxamide (IVl) was first synthesized by Marsico and Goldman in 1965,[3] and compounds of this type were found to be useful in recent syntheses of various 3,9-dialkylpurine derivatives.[4] These authors obtained IVl in 54% yield with a minor amount of 1-methyl-5-(methylamino)imidazole-4-carbonitrile (IIIl) by heating N,N-diethyl-3,9-dimethyladeninium iodide (Ig) in 1 N aqueous sodium hydroxide at 100° for 2 hr. They suggested that IIIl had resulted from the initially formed N,N-diethyl-1-methyl-5-(methylamino)imidazole-4-carboxamidinium (type II·H^+), and hydrolysis of IIIl then produced IVl. We have investigated the alkaline hydrolysis of N,N,3,9-tetraalkyladeninium halides (I) and wish to describe here a general synthesis of 1-alkyl-5-(alkylamino)imidazole-4-carboxamides (IV).

When Ig was treated under conditions similar to those employed by Marsico and Goldman,[3] IVl was obtained in 73% yield. Brief treatment (15 min) of Ig under the same conditions afforded IIIl in 10% yield as well as IVl (68% yield). The nitrile IIIl gave IVl in 58% yield with a 26% recovery of IIIl on brief treatment.

N,N,9-Triethyl-3-methyladeninium iodide (Ih)[5] underwent hydrolysis similarly to give

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3) J.W. Marsico and L. Goldman, *J. Org. Chem.*, **30**, 3597 (1965).
4) *a*) T. Itaya and K. Ogawa, *Heterocycles*, **6**, 965 (1977); *b*) *Idem*, *Tetrahedron Lett.*, **1978**, 2907; *c*) K. Ienaga and W. Pfleiderer, *ibid.*, **1978**, 1447.
5) T. Itaya, K. Ogawa, H. Matsumoto, and T. Watanabe, *Chem. Pharm. Bull.*, **28**, 2522 (1980).