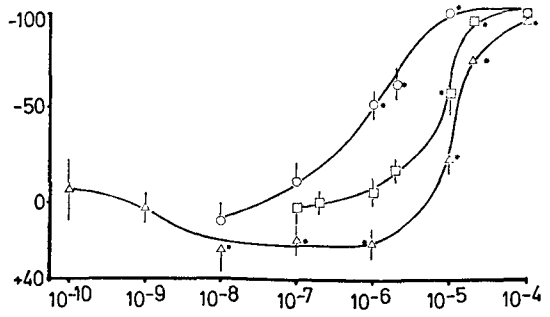

FIG. 1. The effects of indomethacin (△), 5,8,11,14-eicosatetraynoic acid (□) and nordihydroguaiaretic acid (○) on arachidonic acid-induced polymorphonuclear leucocyte aggregation. Values represent the mean of between 6 and 16 determinations and are shown with standard errors. $*P < 0.05$ (Student's *t*-test). Ordinate: % changes compared with arachidonic acid control. Abscissa: molarity.

preferentially from an interference with lipoxygenase enzymes. PMNs may aggregate in response to AA as a result of production of biologically active products of lipoxygenase enzymes (Goetzl & Sun 1979), such as hydroperoxy or epoxide intermediates, or hydroxy fatty acids, e.g. 5-hydroxyeicosatetraenoic acid. These metabolites may play important roles in the biological effects resulting from the metabolism of AA by mammalian cells. The enhancement of the aggregatory response observed with low doses of indomethacin may be due to enhancement of lipoxygenase pathway activity as has been previously reported for this drug (Hamberg & Samuelsson 1974). AA-induced aggregation of PMNs may prove to be a simple and reproducible bioassay for lipoxygenase enzyme activity.

October 25, 1979

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Sustained release of sulphamethizole from agar beads

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A possible use of natural polymers such as konjac, a glucomannan, in dosage form design for sustained release has been examined (Nakano et al 1978). Some of the polysaccharides are known to form gels in which drugs may be incorporated. Agar has been used as culture media in microbiology and agarose, which is a a purified form of agar, has been employed extensively in separation and purification in biochemistry (Hjertén 1964). Agarose beads have recently been examined for possible use in haemoperfusion (Lösgen et al 1978).

In the present study, a possible use of agar for sustained release of sulphamethizole has been examined. The choice of agar rather than agarose is largely due to economic reasons. Agarose is still expensive as an ingredient in dosage forms for commercial production, but it was also studied in order to compare the release characteristics with those of agar. The choice of sulphamethizole as a drug is based on its short plasma half-life and small extent of metabolism (Triggs et al 1975) as well as the availability of reliable analytical methods.

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Sulphamethizole has been used as a urinary disinfectant because most of the dose is excreted in urine in unchanged form. Because of its short half-life in plasma, however, it has to be repeatedly administered in order to maintain the effective concentration in urine. Therefore, a sustained release dosage form may be beneficial.

Materials. Powdered agar was of first grade, Japanese Industrial Standard, purchased from Wako Pure Chemical Industries, Osaka. Water content and total ash as determined according to the test procedures in Japanese Pharmacopoeia were 16·7% and 2·45% (dry weight basis), respectively. Agarose with a labelled gel strength of 600 g cm^{-2} was obtained from Dojin Lab., Kumamoto. Sulphamethizole was of Japanese Pharmacopoeia grade from Eisai Co., Tokyo. Diethyl ether, cyclohexane, light petroleum (b.p. 30–60 °C), hydrochloric acid, potassium chloride, sodium dihydrogen phosphate and disodium hydrogen phosphate were of reagent grade and used as received whereas ethyl acetate, acetone, dioxane, and ethanol were distilled before use.

Preparation of beads. To powdered agar (1·5 g) in an Erlenmeyer flask was added water to make 20 ml and the suspension was heated to 90–95 °C in a waterbath until the agar was completely dissolved. The solution was subsequently cooled to about 70 °C and 2 g of sulphamethizole was suspended in the sol as homogeneously as possible by vigorous mixing. The drug suspension was then quickly taken up into a 3-ml plastic syringe and expelled onto the top of an organic solvent in a 100-ml graduated cylinder which was cooled by immersion in ice-water (Fig. 1). Seven organic solvents were examined as solidification media for the agar sol including ethyl ether which has been used by Bengtsson & Philipson (1964). Spheres were formed and solidified when the sol suspension came into contact with the cold organic solvent. Beads were subsequently separated from the solvent by filtration and were either stored on a sheet of wet filter paper in a capped Petri dish (undried beads) or dried under vacuum for 24 h at room temperature (20 °C) (dry beads). The preparation of agarose beads was carried out in the same way.

Seven solvents with varying polarities were used as solidification media. The water-immiscible solvents: ethyl acetate, ethyl ether, cyclohexane, and light petroleum gave spheres whereas the water-miscible solvents: acetone, ethanol, and dioxane tended to give irregular (oval or coin) shapes. The agar sol was broken down when cold water was used. Solvents were cooled in order to effect faster solidification and minimize loss of the drug due to partitioning. From the water-immiscible solvents, ethyl acetate was selected for further preparation of the beads because of its practical lack of toxicity (Smyth et al 1962). When agar sol containing suspended drug was extruded from a syringe through a hole, 2·15 mm i.d., beads with a diameter 5·0 ± 0·4 mm (n = 20) were obtained. The drug content of the beads was determined by measuring the drug concentration of the release medium at pH 7·4 after it had been released from the beads (24 h). Twenty beads contained about 100 mg of drug. When the wet beads were dried under vacuum at room temperature, most of the water was removed during the first 4 h, but beads were usually dried for 24 h to ensure practical dryness. When twenty beads, weighing 994·4 mg, were dried for 24 h, the weight was reduced to 161·9 mg. The size of the beads was also reduced to 3·0 ± 0·3 mm in diameter.

Measurement of release rates. Twenty beads containing about 100 mg of sulphamethizole were added to 80 ml release medium at 37 °C in a 125 ml wide-mouth bottle in a constant temperature waterbath. The following three release media were employed: Clarke & Lubs HCl-KCl solution at pH 1·0, 0·1 M phosphate buffer at pH 7·4 (McKenzie 1969), and distilled water. The suspension was stirred by a magnetic stirring bar (4 cm long) by means of a submersible magnetic stirrer (Acrobat stirrer, MS Instruments, Osaka) at a rate of about 250 rev min^{-1}. At predetermined intervals, 0·5 ml portion of the medium was taken and diluted with 0·05 M Na_2HPO_4 before absorbance measurements at 262 nm. Release studies were made in duplicate and the average values were plotted. In separate experiments, swelling patterns of the gel were obtained by measuring diameters of twenty beads at predetermined intervals.

Release profiles. Release profiles as well as a dissolution profile of sulphamethizole in the acidic medium are shown in Fig. 2. The release rate of the drug from the dry agar beads was slightly smaller than that from the undried beads and much smaller than the dissolution rate of the drug from pure drug powder. Thus sustained but complete release from the agar gel was observed. Agar gels were thus demonstrated to serve as barriers to liberation of the drug. Slower release from the dry beads than the undried beads may be attributed to a

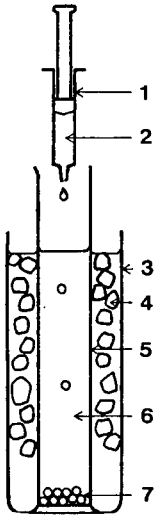

FIG. 1. Schematic drawing of the apparatus used for preparation of agar beads. 1. syringe, 2. hot agar sol with suspended drug particles, 3. wide-mouth bottle, 4. ice-water, 5. graduated cylinder, 6. cold ethyl acetate, 7. agar beads.

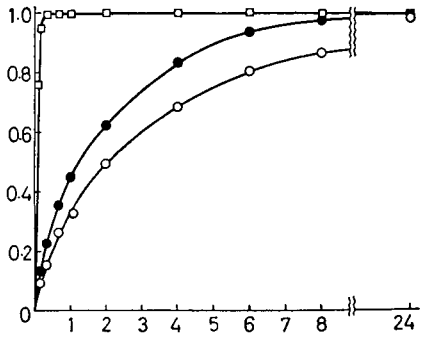

FIG. 2. Release profiles of sulphamethizole from dry (○) and undried (●) beads compared with a dissolution pattern from pure drug powders (□) at pH 1·0 and 37 °C. Ordinate: fraction of the drug liberated. Abscissa: time (h).

smaller initial size due to slow imbibition of water. The size of the beads grew with time from 3·0 mm to 3·9–4·3 mm during the first 1–3 h (Fig. 3) and remained in the same size for the rest of the experimental periods, never reaching the size before drying (5·0 mm).

As shown in Fig. 4, the release rate of sulphamethizole from the dry beads in distilled water was smaller than that in the acidic medium whereas the release rate in the medium at pH 7·4 was greater than that in the medium at pH 1·0. These observations may be rationalized by the solubility differences of the drug in the release media. The pK_a values of the drug have been reported to be 2·20 and 5·45 (Bell & Roblin 1942). Thus the drug is

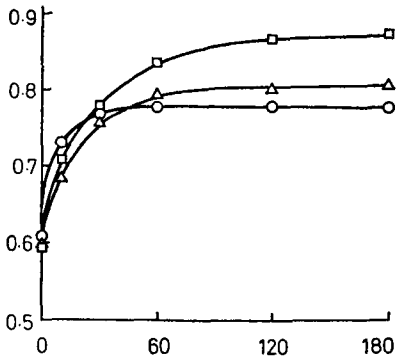

FIG. 3. Swelling patterns of the dry agar gel in three media at 37 °C. △ = pH 7·4, ○ = pH 1·0, □ = distilled water. Ordinate: relative gel size = diameter at time t/diameter before drying. Abscissa: time (min).

expected to exhibit minimum solubility at pH 3·83 and the solubility increases in both directions as pH is lowered or raised from this pH value. Since distilled water exhibited a pH value of around 5, the solubility of the drug in distilled water is expected to be much smaller than that at pH 1·0. Since pH 7·4 is 2 pH unit away from the pKa_2 whereas pH 1·0 is only 1·2 pH unit away from pKa_1, the solubility of the drug is expected to be greater at pH 7·4 than at pH 1·0. These expectations have been substantiated by solubility measurements. The solubility of the drug at 37 °C was 0·819, 12·0 and 141 mg ml^{-1} in distilled water, acid solution at pH 1·0, and in the buffer at pH 7·4, respectively.

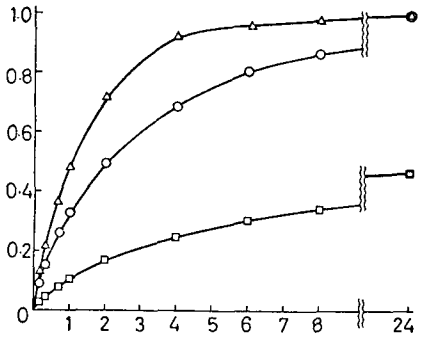

FIG. 4. Release profiles of sulphamethizole from dry agar beads in three media at 37 °C. Symbols as in Fig. 3. Ordinate: fraction of the drug released. Abscissa: time (h).

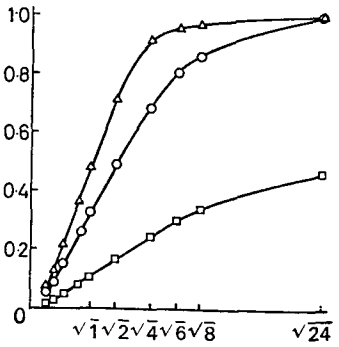

FIG. 5. Plots of the fraction of sulphamethizole released against square root of time. Symbols as in Fig. 3. Ordinate: fraction of the drug released. Abscissa: time $\frac{1}{2}$ ($h^{\frac{1}{2}}$).

When the fraction of the drug released is plotted against square root of time, the straight line relationship was obtained during the initial period (Fig. 5). The square root of time dependency of the fraction of drug released (Higuchi 1963) in hydrophilic gels has been observed in the release profiles of dibucaine, a local anaesthetic, from konjac, a glucomannan, gels (Nakano et al 1978). Similar observations have been made earlier in the drug release from compressed hydrophilic matrices (Lapidus & Lordi 1968). Deviation from the straight line during the latter period of release may be attributed to accumulation of the drug in the release media (non-sink condition) and to the theoretically expected deviation from linearity as the fraction of drug released increases (Cobby et al 1974). The release profile of the drug from the undried agarose beads (not shown in the figure) was not very different from that from the undried agar beads. Thus diffusivity of the drug through the agarose gel is likely to be essentially the same as through the agar gel.

The above results demonstrate that agar is useful for the preparation of beads that exhibit sustained release of the drug. A possible advantage of the agar beads over conventional sustained release formulations is complete release of the drug from the beads, since some of the commercial sustained release preparations have been reported to fail to release all of the drug in the preparation (Hollister et al 1970).

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LETTER TO THE EDITOR

On an in vitro method of simulating drug release from viscous eye drops

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In the report on 'an in vitro method of simulating drug release from viscous eye drops in rabbit and man' (Melis-Decerf et al 1979), the experiment is based on an incorrect interpretation of the relationship of the blink and drug penetration into the eye.

There is a marked difference between the blink rates of man and rabbit (about every 10 s and 15 min respectively). As lid movement is involved in recoating the cornea with drug molecules associated with conjunctiva, the greater the blink rate the greater the probability that the drug will penetrate the cornea rather than be absorbed across the conjunctiva or lost via tear outflow. But the blink *speed* has little, if any, relationship to transcorneal drug penetration. Furthermore as the duration of the blink is a small fraction of the interblink period (assume the blink duration of 0·05 s—an interblink period of 10 s and 15 min for man and rabbit respectively) the tear film is undisturbed by lid movement for more than 99% of the time. Therefore the rationale for constructing a device that can continuously rotate test solutions in a cylinder at a speed similar to that of the lid during its *occasional* excursion over the cornea seems at fault, as also do the authors' assumptions that the rotational speed and that of the fluid at the inner membrane surface are identical and that the device mimicks the shearing action of the lid margin.

July 10, 1979

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