The effects of both the (+)- and (−)-enantiomers of VII were reversed by P2S in both tests in the same manner as the effects of any of the ethyl *S*-alkyl-methylphosphonothioates. It is noteworthy that although the (+)- and (−)-isomers of VII inhibit acetylcholinesterase at different rates and substantially faster than the (+)- and (−)-isomers of the *S*-alkyl methylphosphonothioates (e.g. I(+) and I(−)), the reactivation profiles of all four compounds were indistinguishable.

The results of the *in vivo* experiments showed marked differences from those obtained *in vitro*. The rank order of the LD50 values did not correspond with the rank order of potency of the compounds in the *in vitro* tests (Table 2). Moreover there was sometimes no significant difference between the LD50 values of an enantiomeric pair even though the (−)-isomer was *in vitro* clearly a more potent anti-acetylcholinesterase than the (+)-isomer. In the case of one compound, ethyl *S*-propyl methylphosphonothioate (I), the (+)-isomer was significantly more toxic than the (−)-isomer in contrast to the results of the *in vitro* results.

In the gastrocnemius muscle, as is shown in Fig. 2, the administration of P2S at a dose of 130 μmol kg⁻¹, during poisoning by the (−)-enantiomer of I, re-established neuromuscular function whereas similar P2S treatment of poisoning by the (+)-enantiomer and the racemate failed to restore neuromuscular function. Compounds II and III showed similar behaviour. In marked contrast, the effects of both the (+)- and (−)-

enantiomers of VII on the gastrocnemius muscle preparation were reversed by administration of P2S.

The difference in response to P2S of the (+)- and (−)-enantiomers of the ethyl-methylphosphonothioates I, II and III was consistent with the preliminary observation that rats poisoned by (+)ethyl-*S*-propyl methylphosphonothioate (I+) failed to respond to P2S—atropine treatment. Thus whereas for I(−), treatment by P2S—atropine under standard conditions (Green, Muir & others, 1977) gave a protection ratio of 8·5 (protection ratio = LD50 of Sarin in treated animals/LD50 of Sarin in untreated animals) the protection ratios of I(+) and I(±) were 2·2 and 1·2 respectively. In marked contrast, but in agreement with the results on the gastrocnemius preparation, P2S—atropine treatment of poisoning by VII(±), VII(+) and VII(−) gave protection ratios of 24, 70 and 55 respectively.

To our knowledge there is no recorded precedent for the difference of the (+)- and (−)-enantiomers of the ethyl-methylphosphonothioates observed *in vivo*. Previous comparisons (Fukuto, 1971) of the enantiomers of alkylphosphonothioate anticholinesterases have been concerned mainly with the insecticidal activity and stereoselectivity of cholinesterases from different sources and so no indications of possible mechanistic *in vivo* differences in animals were obtained. That metabolic activation of the (+)-isomers occurs, is an attractive proposition.

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The effects of storage upon *in vitro* and *in vivo* characteristics of soft gelatin capsules containing digoxin

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Encapsulation of a solution of digoxin in soft gelatin is associated with greater bioavailability than from an aqueous solution (Johnson, Bye & others, 1976) or from the solution used to fill the capsules (Mallis, Schmidt & Lindenbaum, 1975). Whereas intestinal absorption of digoxin from tablets is incomplete and

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variable between individuals (Johnson & Bye, 1975), absorption from soft gelatin capsules is virtually complete and shows less between-subject variation (Johnson & others, 1976). The mechanism of the enhanced absorption from such capsules is unclear, but seems likely to be related to either the integrity or chemical composition of the capsule wall. The possibility that altered characteristics of the capsule wall might occur under differing conditions of storage, and

that such changes might be reflected in altered bio-availability, has been examined.

The filling solution for all batches of capsules contained digoxin in a concentration of 950 μg ml^{-1} in 90% w/w polyethylene glycol 400, 6% w/w ethanol, 3% w/w propylene glycol and 1% w/w water. Batches of capsules each containing 0·2, 0·1 and 0·05 mg of digoxin were produced for experimental use by the Rotary Die Process in the United Kingdom factory of R. P. Scherer Ltd.

The capsules were assayed both in bulk and individually using an automated modification of the fluorimetric method for the determination of digoxin described by Wells, Katzung & Meyers (1961). Solution rate was determined by a modification of the method described in the 1975 addendum to the British Pharmacopoeia 1973 using the number of capsules equivalent to 1 mg digoxin, and 1 litre of 0·6% hydrochloric acid (pH 1·2) as the dissolution medium. Capsules were also examined for hydrolysis products of digoxin by thin-layer chromatography.

Samples from the batches of each dosage form were stored at 5, 25 and 37° and re-examined after 1, 3, 6 and 10 months. Ten months after production of the initial capsules, further batches of capsules were manufactured to the same formula by the same encapsulation procedure. These batches were examined by the analytical procedures applied to the earlier batches.

Four male and two female healthy volunteers received single doses of 0·6 mg digoxin on three occasions, with at least 14 days between occasions. Their ages ranged from 27 to 34, and weight from 60–96 kg. None had a past history of serious illness, and none was taking medications other than oral contraceptives.

Following pharmaceutical evaluation of the stored and recently produced batches of capsules, it was decided to study the 0·05 mg dosage form *in vivo*. On separate occasions, each subject received 12 capsules of the fresh batch, the initial batch stored at 5° for 10 months, or the initial batch stored at 37° for 10 months, the sequence of administration being randomized by a Latin-square design. Capsules were admini-
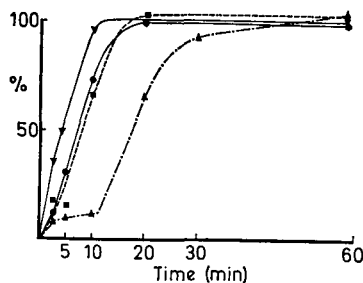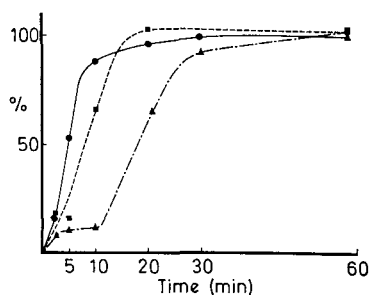

FIG. 2. Comparison of solution rates of 0·05 mg digoxin capsules selected for *in vivo* study. ●—● Freshly prepared capsules. ■—■ Capsules stored at 5°. ▲—▲ Capsules stored at 37°. Abscissa—% digoxin in solution.

stered at 9 a.m. after an overnight fast. Tap water, 100 ml, was taken with the treatments, but nothing else was allowed by mouth for 3 h. Blood samples were obtained via a Braunula cannula in an arm vein at 0·5, 0·75, 1·0, 1·25, 1·5, 2, 3, 5 and 7 h, and urine collected in 24 h periods for 6 days after ingestion of each treatment.

Plasma was quickly separated from blood samples and stored at 4°. Aliquots of urine were obtained and similarly stored as soon as available. Digoxin was determined in all samples in triplicate by radioimmunoassay using the commercially available Lanoxitest-gamma kit (Wellcome Reagents Ltd), in which the tracer is an iodinated tyrosine derivative of digoxin. Standards and reference human plasma or urine were included in each assay run. Statistical significance of differences was assessed by parametric analysis of variance.

No significant differences were observed in mean content, between-capsule variation, or thin-layer chromatographic examination throughout the storage period at all test temperatures, or between the freshly manufactured capsules and the stored product. How-
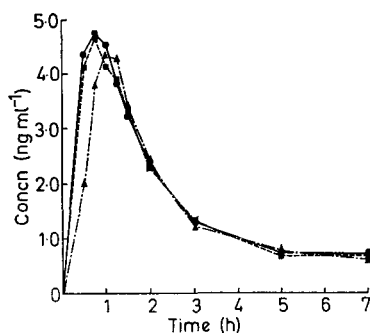

FIG. 1. Effect of storage at various temperatures upon solution rate of 0·05 mg digoxin capsules. ▼—▼ Initial capsules. ■—■ Stored at 5°. ●—● Stored at 25°. ▲—▲ Stored at 37°. Abscissa—% digoxin in solution.

FIG. 3. Curves of mean plasma concentration in 6 subjects after single doses of 0·6 mg digoxin administered as fresh or stored 0·05 mg capsules. ●—● Freshly prepared capsules. ■—■ Capsules stored at 5°. ▲—▲ Capsules stored at 37°. Abscissa—Plasma digoxin concentration (ng ml^{-1}). Ordinate—Time (h) after dose.

Table 1. *Maximal effects of storage upon solution rate profiles of 0·1 and 0·2 mg capsules.*

Min	0·1 mg capsules				0·2 mg capsules			
	2·5	5	10	20	2·5	5	10	20
Initial	8	96	108	—	10	97	104	—
10 months at 37°	0	12	68	106	0	10	103	—

ever, the solution rate noticeably decreased in all samples stored at 37°, the most marked effect being observed in the 0·05 mg capsules (Fig. 1). For this reason stored capsules containing 0·05 mg were selected for comparison against a fresh batch, the solution profiles being illustrated in Fig. 2.

Additional experimental production batches of 0·1 and 0·2 mg capsules were studied, but the maximal effect seen with these capsules was less than for the 0·05 mg capsules chosen for *in vivo* study (Table 1).

Profiles of mean plasma digoxin concentration after administration of each treatment are compared in Fig. 3. Only at 30 min was any significant difference observed. At this time, the mean plasma concentration after ingestion of capsules stored at 37° was lower than for the other two treatments ($P < 0.05$). This reflected a tendency, which was non-significant ($P > 0.05$), for the mean plasma concentration to peak later after ingestion of the capsules stored at 37°. Mean times of peak concentrations were 0·73 h (fresh), 0·75 h (5°) and 0·96 h (37°). However, as shown in Table 2, mean peak concentrations were similar following each treatment ($P > 0.05$) and area under plasma concentration curve determinations were also not significantly different between treatments ($P > 0.05$). Urinary recovery of digoxin following the three treatments showed no significant difference ($P > 0.05$).

It has been assumed that digoxin must dissolve in aqueous intestinal fluids before it can be transported across the intestinal mucosa. For tablets, the extent of absorption was found to be related to the rate at which the contained digoxin dissolved in water (Lindenbaum, Butler & others, 1973; Johnson, Greer & others, 1973) and at best tablets could only provide bioavailability equivalent to that of an aqueous solution of digoxin (Johnson & Lader, 1974). Solution rate has subsequently been recognized as the best available *in vitro* measure of the quality of digoxin tablets.

Table 2. *Plasma and urine results.*

	Fresh capsules	Storage at	
		5°	37°
Peak plasma digoxin concentration (ng ml⁻¹)			
Mean (n = 6)	5·3	5·4	4·8
s.e.m.	0·36	0·35	0·37
Area under plasma concentration curve over 7 h (ng ml⁻¹ h)			
Mean (n = 6)	11·9	11·6	10·7
s.e.m.	0·92	0·79	0·79
Urinary excretion of digoxin (μg) over 6 days after ingestion			
Mean (n = 6)	257·1	258·6	242·0
s.e.m.	16·1	9·2	11·1

It was to be expected that an encapsulated form of digoxin in solution would be of high bioavailability. However the demonstration (Mallis & others, 1975; Johnson & others, 1976) of enhanced bioavailability by comparison with digoxin solution was surprising and is unexplained. It is known that digoxin can be inactivated by gastric acid (Beerman, Hellström & Rosen, 1973; Kuhlmann, Abshagen & Rietbrock, 1973), and encapsulation might offer some protection against this effect.

As the nature of the enhanced bioavailability of encapsulated solution of digoxin is unknown, any change in the characteristics of the capsule wall could be of considerable significance. It was demonstrated that after storage, capsules may take longer to release their contents. This reduced fragility of the capsule wall was demonstrated most clearly for the smallest capsules (0·05 mg) when stored at 37°. The dissolution profile was only altered in its early phase, and all contained digoxin was in solution in the dissolution apparatus by 60 min. Some evidence of delayed release of digoxin was also observed *in vivo*, in that the development of peak plasma concentration appeared slightly delayed (Fig. 3). However, there was no evidence that the extent of absorption of digoxin was reduced. It may be concluded that no important clinical effect is associated with the decreased solution rate after storage under adverse conditions of temperature.

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