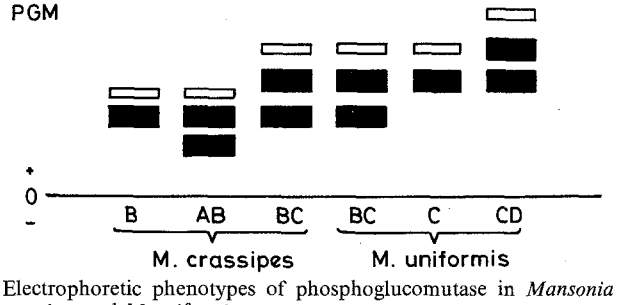
The mosquitoes used for the present study were reared from larvae collected from the wild in Peninsular Malaysia. Adult mosquitoes were used for horizontal starch-gel (12% Sigma hydrolyzed starch) electrophoresis, employing the 'TEMM' buffer system and the enzyme visualization method of Spencer et al. with slight modification[6].

A total of 4 codominant *Pgm* alleles are found in the present samples of *M. crassipes* and *M. uniformis*. As in most culicine mosquitoes, each allele in *M. crassipes* and *M. uniformis* determines a 2-band electrophoretic pattern (fig.). Of these alleles, two (Pgm^B and Pgm^C) are common to *M. crassipes* and *M. uniformis*, while Pgm^A appears to be present only in *M. crassipes* and Pgm^D only in *M. uniformis*. The allele frequencies in *M. crassipes* are $Pgm^A = 0.068$, $Pgm^B = 0.898$ and $Pgm^C = 0.034$, while those in *M. uniformis* are $Pgm^B = 0.045$, $Pgm^C = 0.932$ and $Pgm^D = 0.023$.

Table 2. Frequencies of PGM phenotypes in wild-caught *Mansonia uniformis*

	Homozygotes			Heterozygotes		
	B	C	D	BC	BD	CD
Observed number	0	38	0	4	0	2
Expected number	0.09	38.22	0.02	3.69	0.09	1.89



Electrophoretic phenotypes of phosphoglucomutase in *Mansonia crassipes* and *M. uniformis*.

Of 6 possible phenotypes, only 3 are found in both species. The distribution of the various phenotypes in *M. crassipes* is summarized in table 1, while that in *M. uniformis* is summarized in table 2. In both species the frequencies are in good accordance with Hardy-Weinberg expectations ($\chi^2 = 0.57$ for *M. crassipes* and $\chi^2 = 0.24$ for *M. uniformis*). It is, however, significant that for the 2 common alleles Pgm^B and Pgm^C, the frequencies are significantly different in these *Mansonia* mosquitoes. Pgm^B allele has the highest frequency in *M. crassipes* while Pgm^C allele has the highest frequency in *M. uniformis*. Each of these alleles controls a phenotype of intermediate mobility in these mosquitoes. This agrees with earlier reports for other mosquitoes that the most frequent allele is generally the one controlling a phenotype with an intermediate electrophoretic mobility[3,4,7]. This phenomenon has been taken as supporting evidence for the idea that protein polymorphism is not primarily influenced by random genetic drift acting on a number of neutral isoalleles[8,9]. The present result, in which the common alleles in 2 related species of *Mansonia* mosquito govern different intermediate phenotypes with the highest frequency, renders further support to this hypothesis.

1 This work is supported in part by a University of Malaya research grant to the senior author.
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Precipitate formation of some sulfonated tetrazolthiomethyl cephalosporins with basic chemicals

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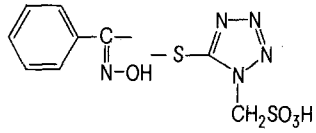
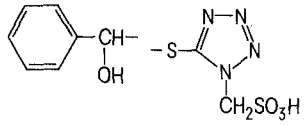
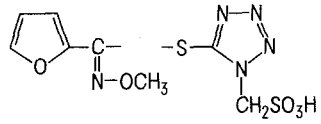
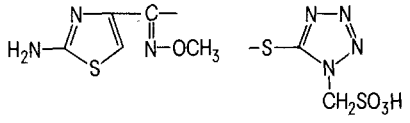
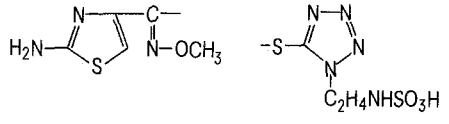
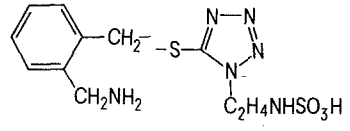
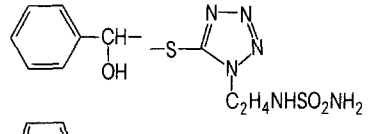
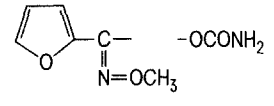
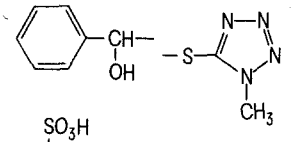
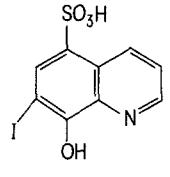
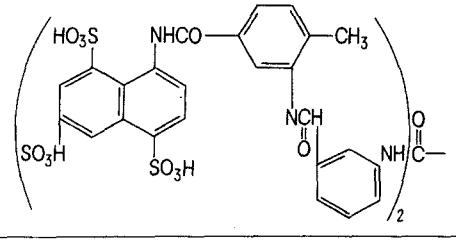
Summary. Cephalosporins containing a thiomethyltetrazole-sulfomethyl or sulfoaminoethyl substituent at the three-position react in aqueous solution with protamine or quinine to form a precipitate. This phenomenon may provide some insight into their pharmacokinetics and modes of action especially as it relates to the high and prolonged serum levels and high degree of serum protein-binding.

Strongly acidic substances, particularly those containing a sulfonic acid and a large electronegative charge form complexes with highly basic materials. This phenomenon gained wide clinical application, as exemplified by the long-acting insulin complexes with either protamine or histone[1], the extremely long acting homidium-suraminate[2] and the depôt-formulation of procaine-penicillin G. The sulfonated and colored azo dyes are known to combine firmly with serum albumin. Evans blue (4 SO_3H groups) is used on the basis of this property for the determination of total blood volume[1]. A similar mechanism is involved in the prompt neutralization of the overdosage symptoms of heparin by the injection of protamine[3,4]. Similar complexing of certain tetrazolsulfonic acid containing cephalosporins with protamine or quinine was observed and is described in this communication.

Materials and methods. 6 sulfonic acid containing cephalosporins were included in this study. 3 non-sulfated cephalosporins (cefuroxime, cefamandole and SK&F 82956) also were included in the experiment as negative controls. 2 sulfonic acid containing drugs, yatren and suramin served as positive controls. The chemical structures are shown in the table.

Compounds were dissolved in deionized water in concentrations of 1000, 500 and 250 µg/ml, whereas protamine sulfate and quinine bisulfate were used in 1% concentra-

Precipitate or cloudiness formation with protamine or quinine of some sulfonic acid-containing cephalosporins in comparison with controls

Name of compounds and/or SK&F No.		Concentration (µg/ml)	Precipitate or cloudiness with	
			Protamine	Quinine
80303		1000 500 250	+ + + + + + + + +	+ + + + + + + + +
Cefonicid (75073)		1000 500 250	+ + + + + +	+ + + + + + + +
81409		1000 500 250	+ + + + + +	– – –
87566		1000 500 250	+ + + +	+ + –
88070		1000 500 250	+ – –	+ + + + + +
82961		1000 500 250	+ + –	– – –
82956		1000 500 250	– – –	– – –
Cefuroxime		1000 500 250	– – –	– – –
Cefamandole		1000 500 250	– – –	– – –
Yatren		1000 500 250	+ + + + + + +	+ – –
Suramin		1000 500 250	+ + + + + + + + + + +	+ + + + + + + + + + +

+ + + + = Bulky precipitate; + = cloudiness; – = no visible change.

tion. To 0.5 ml of the solution of the compounds, 0.15 ml of the protamine or quinine solutions were added and the presence of precipitate or turbidity formation was recorded.

Results and discussion. The chemical structures along with the results obtained are shown in the table. The methylsulfone-tetrazole cephalosporins tended to form more abundant precipitate with protamine or quinine than did the ethylaminosulfonic acid compounds. The non-sulfated cephalosporins (cefuroxime, cefamandole, SK&F 82956) formed neither precipitate nor cloudiness. The number of sulfonic acid groups as well as the substituents at the 7-position seems to influence the bulkiness of the precipitate. This complexing or combining phenomenon with precipitate (cloudiness, opalescence) formation may be a visible indication (in contrast to soluble complexes) of the in vivo binding capacity of these cephalosporins. Cefonicid (SK&F 75073)[5] and SK&F 80303[6] are highly serum protein bound with high and prolonged blood levels following parenteral administration[7]. The other cephalosporins studied, which do not form precipitates, have much lower serum protein binding capacity and lower and shorter serum levels. The concentration of compounds in this in vitro test is obviously higher than the obtainable therapeutic serum levels, nevertheless, the combining property of these agents with basic plasma constituents can be simulated in this test model. The complex formed by protamine or quinine with cefonicid or SK&F 80303 is insoluble in water and has biological activity after washing and vacuum-drying. This test model may prove useful in studying mechanisms of the pharmacokinetic and chemotherapeutic behavior of long-acting tetrazole-sulfonic acid cephalosporins and other chemical agents[9].

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9 Since the submission of the manuscript of this communication, additional and basically similar results were obtained with histone (calf thymus type II) and the recently discovered sulfamate group (–N.SO_3H) - containing novel monocyclic β-lactam antibiotic sulfazecin[8].

Cell cycle patterns of thymidylate synthetase and 5,10-methylenetetrahydrofolate polyglutamates in cultured mouse hepatoma cells[1]

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Summary. The regulation of thymidylate synthetase activity was investigated throughout the first cell cycle after release from an isoleucine block in synchronous cultures of mouse hepatoma (Hepa) cells. Activity in cell extracts increased with the onset of S phase and the increased activity was attributed to a parallel increase in enzyme concentration as determined by titration with tritiated fluorodeoxyuridylate. The polyglutamate chain length of reduced folate cofactors, which could also influence thymidylate synthetase activity, was unchanged.

Thymidylate synthetase (methylenetetrahydrofolate: 2'-deoxyuridine-5'-monophosphate C-methyltransferase; EC 2.1.1.45) catalyzes the reductive methylation of deoxyuridylate (dUMP) to form thymidylate (TMP). The reaction is the one de novo source of dTMP required for DNA synthesis and because the activity of the enzyme is very low in most tissues, it has been suggested that the reaction may be a rate-limiting step in DNA synthesis[2,3]. Due to this pivotal role in DNA synthesis, thymidylate synthetase is a target enzyme in cancer chemotherapy[4] and its regulation during the cell cycle is therefore of considerable interest.

Thymidylate synthetase activity increases dramatically in rapidly proliferating versus stationary cells[5–7] and the specific activity has been shown to increase at the beginning of S phase[8]. Several modes of regulation have been suggested including changes in: a) enzyme synthesis, b) substrate availability, and c) enzyme affinity for the available substrate. Increased specific activity during S phase in mouse 3T6 fibroblasts seems to be controlled at the level of transcription[9]. Neither the concentration of dUMP[10,11] or the monoglutamate form of the folate cofactor[12] appear to limit enzyme activity; however, it has been suggested that changes in the polyglutamate forms of methylenetetrahydrofolate might influence in situ activity[12]. The affinity of thymidylate synthetase is greater for longer chain length polyglutamates[13–17].

The present study involves the stoichiometric titration of thymidylate synthetase in cell extracts[18] and the electrophoretic identification of polyglutamate chain lengths of 5,10-methylenetetrahydrofolate complexes[19] to investigate the influence of enzyme level and polyglutamate chain length on thymidylate synthetase activity during the cell cycle of cultured mouse hepatoma cells.

Materials and methods. All chemicals and materials except those listed below were purchased from Sigma. [6-^{3}H]FdUMP (18 Ci/mmole) and [5-^{3}H]dUMP (18 Ci/mmole) were purchased from Moravek Biochemicals. Folic acid was reduced to tetrahydrofolate by the method of Davis[20] and converted to the methylene derivative by the addition of 75 mM formaldehyde for storage at –70 °C.

The cell line, Hepa, was maintained in culture and synchronized by isoleucine deprivation as previously described[21]. Cell extracts were prepared by a freeze/thaw procedure[22] and aliquots were used for the determination of thymidy-