A. J. Glazko and Parke Davis Corporation for the gift of *p*-hydroxyphenylhydantoin.

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Adenosine uptake by erythrocytes of man, rat and guinea-pig and its inhibition by hexobendine and dipyridamole

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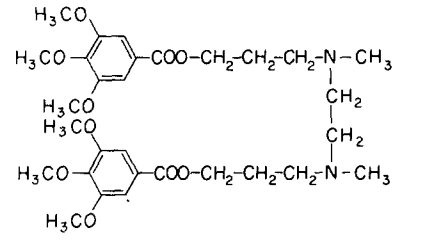
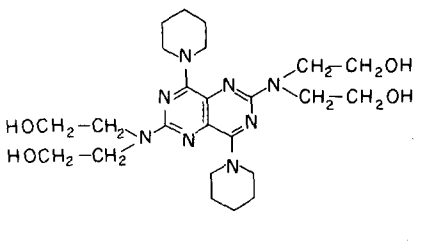
Adenosine is rapidly eliminated from the plasma by the blood cells [1], by the heart [2, 3], and by the lungs [4]. In the guinea-pig, this elimination is very sensitive to dipyridamole and hexobendine. Both drugs exert their inhibitory effect by decreasing the permeability of the cell membrane for adenosine. In the rat, however, the uptake of adenosine into the lungs is about 100 times less sensitive to dipyridamole and hexobendine than in the guinea-pig, while the uptake into the heart is not inhibited at all by these drugs [5, 6].

The uptake of adenosine into the erythrocytes of the guinea-pig, rat and man and the inhibition of this uptake by dipyridamole and hexobendine should provide some information about the sensitivity of adenosine elimination to both drugs in man in comparison with the cited animals.

Blood was collected from the right ventricle of guinea-pigs and rats and from the cubital vein of men. 0·05 mg/ml heparin was added. After centrifugation at 3500 rev/min the supernatant plasma was replaced by ice-cold saline. This procedure was repeated four times. Two ml of the erythrocyte





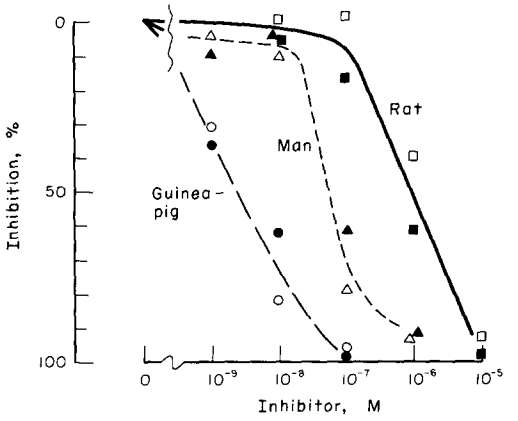


Fig. 1. Inhibition of ^{14}C-adenosine net uptake into erythrocytes of guinea-pigs (○●), men (△▲) and rats (□■) by different concentrations of hexobendine (open symbols) and dipyridamole (closed symbols).

Table 1. Effect of hexobendine and dipyridamole on the uptake of ^{14}C-adenosine by erythrocytes of guinea-pigs, men and rats

Species		Inhibitor (M)	After incubation 0	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	Before incubation
Guinea-pig	Erythrocytes	Hexobendine	36·9* ±1·2	30·7 ±1·9	20·7 ±0·4	17·6 ±1·6	15·3 ±1·0	16·3 ±1·9	14·7 ±1·2	17·0 ±2·0
	Supernatant		48·8 ±2·1	59·0 ±1·1	79·1 ±1·3	82·8 ±5·0	88·0 ±2·6	86·6 ±1·3	89·1 ±1·5	86·6 ±2·5
	Erythrocytes	Dipyridamole	36·0 ±2·0	29·5 ±2·0	24·5 ±2·0	17·5 ±2·0	15·5 ±2·0	—	15·0 ±3·3	17·5 ±4·5
	Supernatant		46·5 ±4·0	52·0 ±5·0	61·5 ±2·0	70·0 ±6·0	75·0 ±2·5	—	76·5 ±1·5	71·0 ±5·5
Man	Erythrocytes	Hexobendine	54·9 ±3·4	53·2 ±3·3	50·5 ±6·2	18·6 ±7·1	12·1 ±1·2	11·3 ±2·0	10·8 ±3·0	9·0 ±1·2
	Supernatant		38·1 ±6·2	41·9 ±5·5	43·8 ±8·1	77·5 ±8·8	89·0 ±3·0	87·9 ±2·7	87·0 ±3·8	89·0 ±4·7
	Erythrocytes	Dipyridamide	53·0 ±6·6	48·7 ±6·2	51·3 ±7·6	26·2 ±15·5	12·7 ±1·3	10·6 ±0·5	8·9 ±1·4	9·3 ±1·8
	Supernatant		39·2 ±8·3	42·7 ±3·8	43·2 ±9·1	71·2 ±19·8	86·7 ±2·9	86·7 ±3·8	88·8 ±5·8	90·0 ±0·8
Rat	Erythrocytes	Hexobendine	45·5 ±2·9	44·8 ±2·0	46·0 ±1·8	46·3 ±1·7	36·9 ±3·1	20·5 ±0·8	16·8 ±1·8	18·0 ±3·0
	Supernatant		17·8 ±1·7	18·5 ±3·5	19·4 ±1·4	19·8 ±1·2	38·4 ±1·2	73·8 ±1·4	82·4 ±1·7	78·7 ±3·8
	Erythrocytes	Dipyridamole	61·1 ±1·5	57·7 ±2·0	59·1 ±0·7	54·4 ±0·7	36·3 ±0·8	21·6 ±2·3	18·5 ±2·2	21·0 ±1·7
	Supernatant		17·0 ±2·9	17·4 ±2·9	16·8 ±0·9	24·8 ±1·2	52·5 ±1·5	78·5 ±1·9	83·2 ±4·6	83·1 ±6·5

* Percentage of added ^{14}C in the erythrocyte fraction and supernatant.
The values are the mean ±S.D. of four individual measurements.

suspension thus prepared was incubated for 5 min with 0·05 ml of a solution, which contained dipyridamole or hexobendine in a final concentration of 10^{-9} to 10^{-4} M. After the addition of 2 μg ^{14}C-adenosine (Radiochemical Centre, Amersham) in 0·2 ml saline the erythrocytes of the rat and the guinea-pig were incubated for 9 min at 17°, the human erythrocytes for 1 min at 7°. The incubation was stopped by adding 0·5 mg dipyridamole in 0·1 ml and cooling almost to freezing point in a methanol bath (−30°). After centrifugation (4500 rev/min, 0°, 1 min) the supernatant was siphoned into another tube. The remaining erythrocytes as well as the supernatant were immediately mixed with 2 ml trichloroacetic acid (5% w/v) and centrifuged again. The amount of ^{14}C in 1 ml of both final supernatants was measured with a Tri-carb Liquid Scintillation Spectrometer (Packard, Model 3002) using a dioxane-scintillator [7].

The amount of ^{14}C measured in the erythrocyte fraction after incubation with ^{14}C-adenosine was 40–60 per cent of the added radioactivity. It was reduced by dipyridamole and hexobendine (Table 1). When the reaction was stopped immediately after addition of ^{14}C-adenosine, 10–20 per cent of the added ^{14}C was found in the erythrocyte fraction. This ^{14}C-adenosine might be included in the small space between the sedimented erythrocytes or absorbed to their surface.

A dose–response curve was prepared by subtracting the amount of ^{14}C in the erythrocyte fraction at 0 min, i.e. stopping the uptake immediately after the addition of ^{14}C-adenosine, from the amount of ^{14}C at the end of the incubation period. The net uptake in the samples without dipyridamole or hexobendine thus calculated was taken as 100 per cent (Fig. 1). A 50% inhibition of ^{14}C-adenosine uptake is seen at 10^{-8} M dipyridamole or hexobendine for guinea-pig erythrocytes, at 10^{-7} M for human erythrocytes and at 10^{-6} M for rat erythrocytes. The human erythrocytes were therefore 10 times more sensitive than those of the rat and 10 times less sensitive than those of the guinea-pig. Further investigations of the uptake, the incorporation and degradation of adenosine by erythrocytes of different species (unpublished observations) have shown that these processes occur much faster in human erythrocytes, even at 7°, than in the guinea-pig and the rat at 17°. Similar results have been reported for adenosine uptake by blood of different species, measuring the uptake velocity at a 30 times higher adenosine concentration [1]. Thus it is possible that the dose-response curve shown in Fig. 1 for human erythrocytes is shifted to higher inhibitor concentrations. The greater steepness of the inhibition curve also confirms the assumption that the onset of inhibition is not seen at the described experimental conditions unless it is completely developed at high concentrations of dipyridamole or hexobendine. Therefore it is supposed that the sensitivity of human erythrocytes is similar to the sensitivity of the erythrocytes of the guinea-pig.

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A basis for the difference in toxicity of methotrexate, aminopterin and methasquin in mice

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The folate analogs, methotrexate (MTX), methasquin (MQ) and aminopterin (AM), vary considerably in their toxic potency in animals [1–10]. The basis for this difference is still not understood. All three derivatives affect DNA synthesis via inhibition of dihydrofolate reductase [10–16]. This inhibition is nearly stoichiometric in each case [10], and any differences shown to occur among these analogs are insignificant in respect to any overt manifestation on toxicity.

In mice, MTX, MQ and AM have a similar qualitative effect [2, 9, 10] on the proliferating crypt epithelium of the small intestine, the most drug-sensitive tissue in this species. The LD_{10} or LD_{50} for MTX, MQ and AM after a single dose, however, is roughly in the order of 18:3:1. Part of the increased toxicity associated with MQ when compared to MTX may be attributed to a lower rate of clearance from plasma [10]. The rate of clearance of MTX and AM, however, are essentially the same [10]. Previous work [10, 17] has also shown that the degree of lethality observed at varying doses of MTX is proportional to the duration of inhibition of DNA synthesis in the small intestine. The duration of inhibition by MTX in this tissue has subsequently been found [18] to depend upon the extent to which drug persists at levels above the dihydrofolate reductase content. In the present study, we have examined the extent of uptake and persistence of free (unbound to enzyme) MTX, MQ and AM in mouse small intestine at the approximate equimolar, equitoxic and therapeutically optimum doses. Our results suggest, as a basis for the relative toxicity of these agents, differences in the degree of persistence of each in this highly susceptible tissue.

BDF_1 (C57BL/6 × DBA_2 male) mice were injected intraperitoneally with drug. At varying times thereafter, animals were sacrificed by cervical dislocation, and the small intestine was surgically removed. The methods used for washing and homogenizing the tissue and extracting drug have been described [10, 17, 18]. The drug content of the heated tissue supernatant was determined by a titration assay with a microbial dihydrofolate reductase [19]. Values given in the results are averages based on determinations with two to four animals. The dihydrofolate reductase content in unheated tissue extracts was obtained by titration with antifolate [13, 19]. MTX and AM were generously supplied by Lederle Laboratories. MQ was obtained as a gift from Parke Davis & Co. When analyzed by enzyme titration, samples of MTX and MQ were essentially pure. The AM sample was only 59 per cent pure as determined by the same assay. An identical percentage was recovered in the inhibitory fraction obtained by chromatography on DEAE-cellulose. AM dosages are expressed as the equivalence in pure drug.

The uptake and loss of MTX, MQ and AM in mouse small intestine after the administration of 3 mg/kg i.p. are

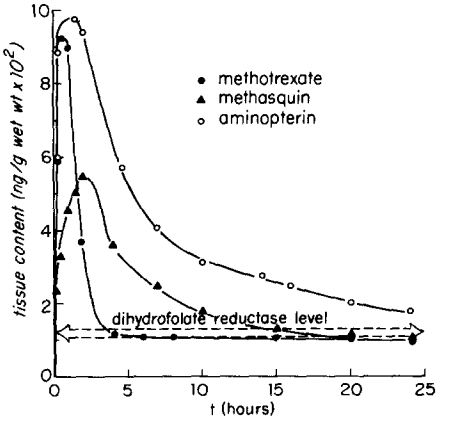

Fig. 1. Drug content of mouse small intestine at varying times after the administration of MTX, MQ or AM at 3 mg/kg i.p. Each point represents an average of two to four animals in two separate experiments.