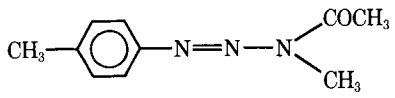

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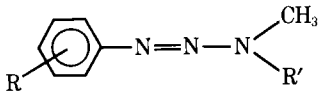

R′ = alkyl

II

alkyl-3-methyltriazenes were reported previously (2, 3). The triazenes were tested against *T. rhodesiense* (Wellcome CT strain) in the mouse (ICR/HA Swiss) (4). Test and control mice were 6 weeks old and weighed 28–30 g. No differences in response between male and female mice have been reported. Each mouse was infected with an intraperitoneal injection of 0.05 ml of a 1:50,000 dilution of heparinized heart blood drawn from donor mice infected 3 days earlier.

Drugs were administered subcutaneously as a single dose in peanut oil 2 hr after injection. Untreated mice died between 4.2 and 4.5 days after injection. Surviving animals were observed for 30 days and mice surviving after this time were considered cured. The surviving mice were not checked for parasitemia. Test data are given in Table I. The activities of some of the compounds against Sarcoma-180 in the mouse are also included.

The activities reported in Table I suggest that triazenes have curative effects against *T. rhodesiense* in mice. However, high doses are required to produce these effects. It has been suggested that similarities exist between some metabolic pathways of the predominant bloodstream form of the African trypanosome and tumor cells (5) and it was shown that a number of anticancer agents are active against *T. rhodesiense* (6). This correlation was also suggested by the data in Table I when a comparison was made of those activities for the compounds on which both types of data were determined. Compounds 2 and 4 are active in both test systems. Compounds 1 and 3 also were reported to be active against murine leukemia L-1210 in the mouse while the *p*-phenyl analog of II (compound 5) is inactive against *T. rhodesiense*, S-180, and L-1210 (7).

Table I—Activity of II against *T. rhodesiense* Infection in Mice

	R	R′	Dose, mg/kg ip	Antitrypanosomal Activity[a]	Anticancer Activity[b]
1	*p*-COOH	CH_3	424	4/5	
			424[c]	5/5	
2	*m*-COOH	CH_3	424	1/5	3.01[d]
3	*p*-NHCO-CH_3	CH_3	424	2/5	
			212	1/5	
			424[c]	2/5	
4	*p*-NO_2	CH_3	424	3/5	3.43[d]
			212	1/5	
			424[c]	3/5	
5	*p*-C_6H_5	CH_3	424	Inactive	Inactive
6	*m*-CF_3	CH_3	424	Inactive	3.18[e]
7	*m*-Cl	CH_3	424	Inactive	3.16[e]
8	*p*-COOH	$CH_2C_6H_5$	424	Inactive	Inactive[e]
9	*p*-COOH	$CH_2C_6H_4$-*p*-CH_3	424	Inactive	3.25[e]
10	*p*-COOH	$CH_2C_6H_4$-*p*-OCH_3	424	Inactive	Inactive[e]
11	*p*-COOH	$CH_2C_6H_4$-*p*-NO_2	424	Inactive	Inactive[e]
12	*p*-COOH	$CH_2C_6H_4$-*p*-CN	424	Inactive	Weakly active[e]
13	*p*-CN	$CH_2C_6H_4$-*p*-CH_3	424	Inactive	Inactive[e]
14	*p*-CN	$CH_2C_6H_4$-*p*-Cl	424	Inactive	Inactive[e]

[a] Cures per five treated animals. [b] Activity is given as $-\log C$; C is the moles/kg required to give an increase life span of 130% of control. Tumor was S-180 in the mouse. [c] Duplicate. [d] From reference 2. [e] From reference 3.

The exact mechanism of the anticancer action of the trianzenes is not known but it is known that the 3,3-dialkyltriazenes undergo extensive metabolic *N*-dealkylation to produce alkylating intermediates (8). This metabolic pathway to activation could be host-mediated or it could also be present in the African trypanosome and be involved in the mechanism of action of the triazenes against this parasite. The antitrypanosomal activities of triazenes are being studied further.

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Comparison of Equilibrium Times in Dialysis Experiments Using Spiked Plasma or Spiked Buffer

Keyphrases □ Equilibrium dialysis—comparison of spiked plasma and spiked buffer □ Protein binding—effect of spiked plasma and spiked buffer on equilibrium dialysis

To the Editor:

When the plasma protein binding of a drug is to be determined using equilibrium dialysis *in vitro*, the drug can be added to either the buffer side or the plasma side of a dialysis cell. Adding drug to the plasma will dilute the plasma proteins if the drug has to be added as a solution. Adding the drug solution to the buffer avoids this difficulty. However, the approach to equilibrium is slower when the buffer is spiked than when the plasma is spiked. Under conditions where the equilibrium conditions can change

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with time, as is seen with quinidine (1), it is important to realize the difference in the time to approach equilibrium between adding drug to plasma and buffer.

For the following discussion, the associating and dissociation rates are assumed to be fast in comparison to the diffusion across the dialysis membrane. Furthermore, it is also assumed that there is the same volume V on both sides of the dialysis membrane, and no net flux of water takes place. In addition, the rate of transfer across the membrane is assumed to be covered by a clearance term Cl_T, which is the same in both directions, and the unbound concentration on each side. The binding is assumed to be linear and no net loss of drug occurs during dialysis.

The rate of change in the buffer side can be expressed by:

$$\frac{dA_B}{dt} = -Cl_T C_B + Cl_T \alpha C_p \qquad \text{(Eq. 1)}$$

where A_B is the amount on the buffer side, α is the unbound fraction in plasma, C_B the buffer concentration, and C_p the plasma concentration of drug. The rate of change in the plasma side is expressed by:

$$\frac{dA_p}{dt} = -Cl_T \alpha C_p + Cl_T C_B \qquad \text{(Eq. 2)}$$

where A_p is the amount on the plasma side.

When the drug initially is placed on the plasma side, the concentration on the buffer side is then described by:

$$C_B = \frac{C_0 \alpha}{1+\alpha}\left[1 - e^{-(k_T\alpha + k_T)t}\right] \qquad \text{(Eq. 3)}$$

where t is time, C_0 initial concentration, and k_T the ratio Cl_T/V.

When the drug initially is placed on the buffer side, the buffer side concentration is described by:

$$C_B = \frac{C_0}{1+\alpha}\left[\alpha + e^{-(k_T\alpha + k_T)t}\right] \qquad \text{(Eq. 4)}$$

In both cases the concentration approaches the equilibrium concentration $(C_0\,\alpha)/(1+\alpha)$ when $t \to \infty$. One can determine from Eqs. 3 and 4 the time that is needed to reach a value that is only a fraction, δ, away from the equilibrium concentration; *i.e.*, the time required to reach a buffer concentration of $[(C_0\,\alpha)/(1+\alpha)]\,(1-\delta)$ if the drug is placed initially on the plasma side and $[(C_0\,\alpha)/1+\alpha]\,(1+\delta)$ if it is placed initially on the buffer side. The ratio of the times to reach these concentration is then:

$$R = \frac{t_B}{t_p} = \frac{\ln\left\{\left[\left(\frac{C_0\alpha}{1+\alpha}\right)(1+\delta)\Big/\frac{C_0}{1+\alpha}\right] - \alpha\right\}}{-(k_T\,\alpha + k_T)} \qquad \text{(Eq. 5)}$$

$$\frac{\ln\left\{\left[-\frac{C_0\,\alpha}{1+\alpha}(1-\delta)\Big/\frac{C_0\,\alpha}{1+\alpha}\right] + 1\right\}}{-(k_T\,\alpha + k_T)} \qquad \text{(Eq. 5)}$$

where t_p and t_B are the times to reach equilibrium when the drug is initially added to the plasma and buffer side, respectively. Cancelling the common terms gives:

$$R = \frac{t_B}{t_p} = \frac{\ln\delta + \ln\alpha}{\ln\delta} = 1 + \frac{\ln\alpha}{\ln\delta} \qquad \text{(Eq. 6)}$$

Equation 6 indicates that the closer to the true equilibrium value one wants to be, the closer the ratio is to unity. The stronger the binding and the larger the deviation from the true equilibrium concentration, the greater the advantage of spiking the plasma side.

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Stability of Heparin and Other Fractions of Glycosaminoglycan Sulfates in Human Digestive Juices

Keyphrases □ Heparin—stability in human digestive juices □ Glycosaminoglycan sulfates—stability in human digestive juices □ Absorption, intestinal—stability of heparin and glycosaminoglycan sulfates

To the Editor:

The oral administration of heparin has been a subject of controversy (1) because of its uncertain absorption in the GI tract and its stability in digestive juices. Its N-sulfate groups are easily hydrolyzed in an acidic medium, with consequent impairment of biological activity. Some authors (2) also affirmed that intestinal flora degrades and metabolizes heparin.

However, according to recent studies (3, 4), some fractions of glycosaminoglycan sulfates, strictly correlated to heparin, exerted antithrombotic and hypolipidemic activities after oral administration. Furthermore, it was proved that intestinal absorption took place when some fractions of glycosaminoglycan sulfates, labeled with fluorescein (5), were administered intraduodenally (6).

Special excipients to suppress ionization of the functional groups of glycosaminoglycan sulfates and, thus, to promote their GI absorption (7) were studied. It was found that, if biopolymer is administered in micellar suspension with monoolein and bile salts, the intestinal absorption of heparin greatly increases with increasing mucosa permeability (8). For example, the rectal administration to rats of heparin and other glycosaminoglycan sulfates in a 2-mg/kg dose in an oily emulsion prolonged the time of coagulation; only 1 mg/kg, administered by the same route, activated the lipoprotein lipase-inducing system (9).

To evaluate if and to what extent human digestive juices can degrade glycosaminoglycan sulfates, we investigated the stability and biological activity of heparin and a standardized mixture[1] of fractions of glycosaminoglycan sulfates, containing a heparin-like substance with a low molecular weight and condroitin sulfate B, in cat and human gastric and duodenal juices.

Glycosaminoglycan sulfates and heparin were incubated at concentrations of 190 and 238 USP/ml, respectively, for up to 3 hr in digestive juices[2] at 37°. To evaluate the in-

[1] Sulodexide.
[2] Gastric juice of the cat was obtained by a Heidenhain gastric pouch. Human gastric juice was obtained from a probe of five volunteers who had previously been given an injection of 3 U of cholecystokinin kg/hr iv.

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