EXPERIMENTAL[1]

Melting points were determined with the Fisher-Johns melting-point apparatus and are corrected.

The procedure for 2,4-dinitrobenzenesulfenamide formation was identical to the one reported (3) except that methylene chloride was used as the reaction solvent. The results are summarized in Table I.

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COMMUNICATIONS

Distribution of Ampicillin Administered Orally in Three Different Forms in Rabbit

Keyphrases ☐ Ampicillin distribution—rabbit ☐ Distribution, rabbit—anhydrous, trihydrate, and metampicillins

Sir:

Many physicochemical factors, like particle size (1), salt form (2), ester form (3), or crystal form (4), can affect the dissolution rate and the pharmacokinetics of antibiotics. Particularly, different plasma levels of antibiotic after administration of anhydrous or trihydrate ampicillin (5) and different biliary levels of antibiotic after administration of ampicillin or metampicillin have been described (6). We have reported the distribution of ampicillin in the rabbit after oral administration of three forms of 6-[D(−)-α-aminophenylacetamido]-penicillanic acid: anhydrous ampicillin (AA), trihydrate ampicillin (TA), and metampicillin (MA) (condensation product of ampicillin with formaldehyde).

Sixty male New Zealand rabbits, 2.8 ± 0.3 kg., were fed on a pellet diet with water *ad libitum* and kept at 21 ± 1° and relative humidity 50 ± 4%. The fasting animals were treated orally in random order with 50 mg./kg. of the ampicillins, the amounts of which were expressed as 6-[D(−)-α-aminophenylacetamido]penicillanic acid, and with a mean particle diameter of 14 ± 7 μ. At random order, the animals were killed by bleeding 0.5, 1, 2, and 4 hr. after antibiotic administration; all specimens of blood were taken with a sterile syringe. The kidney, liver, stomach, duodenum, lung, brain, heart, spleen, and muscle were aseptically homogenized for 5 min. with a 0.2 *M* buffer phosphate solution, pH 7; the homogenates were centrifuged for 10 min. at 3000 r.p.m., and assays were performed on the supernatants. The assays of ampicillin (in terms of micrograms per milliliter or micrograms per gram) were carried out on the same day the test was made.

Ampicillin levels were assayed by the cup-plate method, with *Bacillus subtilis* FB27 as the test organism. Samples of plasma, supernatants, and antibiotic standards were diluted when necessary in phosphate buffer, pH 7; several tests were also made with ampicillin-free plasma and supernatants, and no antibacterial activity was detected. The standard solutions were made with plasma or supernatants from untreated animals.

No statistical differences were evident (Table I) in the distribution of the ampicillin administered as anhydrous ampicillin, trihydrate ampicillin, or condensation product with formaldehyde. Sutherland and Robinson (7) also found that the activities demonstrated by the condensation products of ampicillin with acetone or formaldehyde corresponded closely with the rates to which these compounds hydrolyzed to ampicillin.

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Table I—Distribution of Ampicillin (Micrograms per Milliliter or Micrograms per Gram) after Oral Administration in the Rabbit of Anhydrous Ampicillin, Trihydrate Ampicillin, and Metampicillin at 50 mg./kg. as 6-[D(−)-α-Aminophenylacetamido]penicillanic Acid

Hours after Antibiotics Administration	Antibiotics[a]	Plasma	Organs: Kidney	Stomach	Duodenum	Liver	Lung	Brain	Muscle	Spleen	Heart
0.5	AA	1.31 ± 0.27	9.32 ± 2.52	20.09 ± 2.37	68.21 ± 9.01	0.42 ± 0.10	0.74 ± 0.17	0.22 ± 0.03	0.65 ± 0.11	0.64 ± 0.09	0.88 ± 0.17
	TA	1.06 ± 0.16	7.57 ± 1.52	19.97 ± 2.72	58.20 ± 6.51	0.58 ± 0.09	0.73 ± 0.06	0.39 ± 0.04	0.46 ± 0.03	0.72 ± 0.12	0.92 ± 0.12
	MA	1.20 ± 0.22	8.12 ± 1.13	25.46 ± 3.78	57.42 ± 5.56	0.34 ± 0.08	0.88 ± 0.10	0.29 ± 0.07	0.59 ± 0.11	0.66 ± 0.10	0.96 ± 0.07
1	AA	2.25 ± 0.29	15.89 ± 2.32	8.69 ± 1.91	18.54 ± 2.02	0.44 ± 0.09	1.94 ± 0.37	0.47 ± 0.11	0.94 ± 0.09	1.26 ± 0.23	1.52 ± 0.20
	TA	2.36 ± 0.34	19.91 ± 3.64	10.39 ± 1.24	22.29 ± 2.41	0.51 ± 0.09	1.86 ± 0.44	0.44 ± 0.04	1.00 ± 0.18	1.79 ± 0.11	1.88 ± 0.31
	MA	2.19 ± 0.38	17.27 ± 2.06	9.70 ± 1.66	21.24 ± 2.62	0.55 ± 0.04	1.67 ± 0.23	0.57 ± 0.12	1.00 ± 0.21	1.33 ± 0.25	1.63 ± 0.29
2	AA	1.16 ± 0.21	10.77 ± 1.62	1.59 ± 0.15	6.82 ± 1.05	0.40 ± 0.12	0.59 ± 0.11	0.26 ± 0.05	0.58 ± 0.06	0.55 ± 0.06	0.59 ± 0.08
	TA	0.95 ± 0.26	10.88 ± 1.33	1.49 ± 0.34	5.30 ± 0.57	0.40 ± 0.11	0.67 ± 0.16	0.32 ± 0.08	0.79 ± 0.25	0.64 ± 0.11	0.53 ± 0.11
	MA	1.11 ± 0.17	8.18 ± 1.10	1.87 ± 0.28	6.29 ± 0.50	0.48 ± 0.09	0.84 ± 0.13	0.22 ± 0.06	0.65 ± 0.08	0.61 ± 0.08	0.68 ± 0.07
4	AA	0.45 ± 0.06	3.66 ± 0.47	0.56 ± 0.10	1.97 ± 0.30	0.06 ± 0.03	0.32 ± 0.07	0.13 ± 0.03	0.42 ± 0.08	0.24 ± 0.07	0.30 ± 0.05
	TA	0.54 ± 0.11	3.80 ± 0.78	0.50 ± 0.07	1.67 ± 0.13	0.12 ± 0.06	0.39 ± 0.13	0.13 ± 0.03	0.40 ± 0.11	0.20 ± 0.03	0.29 ± 0.04
	MA	0.38 ± 0.04	3.28 ± 0.67	0.68 ± 0.12	2.45 ± 0.34	0.11 ± 0.04	0.38 ± 0.07	0.16 ± 0.04	0.47 ± 0.08	0.31 ± 0.06	0.37 ± 0.05

[a] AA = anhydrous ampicillin; TA = trihydrate ampicillin; and MA = metampicillin. $n = 5$.

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Translocation of Ligand Conformational Free Energy in Receptor Activation: A Possible Functional Role of Conformational Isomerism in Drug Action

Keyphrases □ Ligand conformational free energy—receptor activation □ Conformation isomerism relationship—biological activity

Sir:

Numerous attempts have been made to define the pharmacophoric conformations of physiologically active molecules (1). Some have postulated that the preferred conformation is the pharmacophoric species (2, 3), while others have concluded that, at least in one case, an energetically unfavorable conformation is bound to the receptor (4–6). On the other hand, Gill (7) implied that more than one conformation may be involved, and Shefter (8) recently pointed out the inadequacies of associating a unique conformation of cholinergic ligands with biological activity. None of these speculations has ascribed a functional role to conformational equilibria in the ligand–receptor interaction.

We wish to propose a model in which *conformational* free energy of the ligand is utilized to perform work in assisting conformational reorganization of the receptor assembly. The concept described is based on the widely accepted hypothesis (9–12) that a specific conformational change of the receptor assembly gives rise to a biological effect.

The model envisages ligand–receptor association and events leading to depolarization and recovery as a cyclic process (Scheme I). The agonist exists as an equilibrium mixture, $A \rightleftarrows B$, of a preferred (B) and a higher energy (A) conformer. It is assumed that A combines with resting receptor component R faster then does B and that $k_1 \gg k_{-1}$. The receptor component is considered to be a regulatory site (11) which is coupled to one or more macromolecular membrane subunits (M). The activated receptor component (R^*) is capable of triggering a conformational reorganization of M to M', thus giving rise to depolarization.

The salient features associated with various phases of the cycle are as follows:

1. The *higher energy* conformer (A) has greater affinity for R when compared to B; the difference in