sis of tetrahydrofolate de novo. Trimethoprim thus inhibits an enzyme of a cyclic pathway, and the overall concentration of intermediates of this pathway can be reduced by sulfonamides. Webb (*3*, p. 501) has noted that in similar cases two such inhibitors might have a combined effect out of all proportion to their individual inhibitions. Such effects could lead to synergism at the inhibitor concentrations where synergism is observed experimentally in growing bacteria.

JAMES J. BURCHALL
Department of Microbiology, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

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Poe's observation (*1*) that sulfonamides weakly inhibit dihydrofolate reductase and influence binding of trimethoprim to the same enzyme led him to propose an alternative explanation for the currently accepted mechanism of synergism between these drugs. While the effects of simultaneous binding to isolated dihydrofolate reductase have to await confirmation, at this laboratory we think that there are several relevant facts which are difficult to reconcile with the mechanism proposed by Poe (*1*).

Several authors have observed that dihydrofolate reductase inhibitors of widely varying structures potentiate sulfonamides to the same extent as does trimethoprim in vitro. The lack of synergism between 2,4-diaminopteroylaspartate and sulfadiazine, which was cited by Poe as being an exception, refers to chemotherapeutic experiments with plasmodia and is readily explained by the fact that this compound, similar to other folate derivatives, does not penetrate bacterial and plasmodial cells, as was also mentioned by Rollo (*2*).

If the data from numerous published isobolograms of trimethoprim-sulfamethoxazole combination are replotted in molar concentrations, one finds that the concentrations needed to produce synergism are in the nanomolar range for trimethoprim and in the micromolar range for sulfamethoxazole (*3*). Hence the sulfonamide concentration is generally four orders of magnitude below that which is needed to obtain any observable synergistic effect in Poe's model. These concentrations are reached in most tissues.

The most difficult fact to reconcile with Poe's proposal is the effect of *p*-aminobenzoic acid (*p*ABA), which in low concentrations completely eliminates any antibacterial activity of the sulfonamide and any synergism with trimethoprim. We observed that the presence of 1 μg of *p*ABA per milliliter ($7.6 \times 10^{-6} M$) completely suppressed potentiation, leaving unimpaired the activity of trimethoprim. This has also been demonstrated in growth-kinetic experiments (*4*). On the other hand, we also found that *p*ABA inhibited by 50 percent the activity of dihydrofolate reductase at a concentration of $1.5 \times 10^{-2} M$ (0.06 m*M* substrate). Hence, if one assumes that Poe's mechanism is correct, micromolar concentrations of *p*ABA would probably have to replace millimolar concentrations of sulfonamide acting on the dihydrofolate reductase. This remains to be proved.

We conclude that in vitro and in vivo the mechanism proposed by Poe (*1*) can hardly be considered as an alternative to what has so far been more adequately explained by the mechanism of sequential blockade.

RUDOLF THEN
Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ch-4002 Basel, Switzerland

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As was stated in (*1*), "Very high concentrations of sulfonamide are required to observe this synergism [between trimethoprim and sulfamethoxazole]." Burchall and Then correctly note a number of situations where dihydrofolate reductase inhibition by sulfa drugs could not be of significance since biological effects were noted at micromolar concentrations of the drugs; this includes Then's data for *p*-aminobenzoic acid. Nevertheless, very high concentrations of sulfamethoxazole [up to $2 \times 10^{-3} M$ (*2*)] are attained in normal trimethoprim-sulfamethoxazole clinical regimens. And, the theory of sequential inhibition does not provide an explanation for synergism between sulfa drugs and trimethoprim noted in sulfa-resistant organisms (*3*); in this case high concentrations of sulfa are used.

Then's suggestion that classical antifolates such as methotrexate and 2,4-diaminopteroylaspartate do not potentiate sulfa action in bacteria and plasmodia because of membrane impermeability is well made.

In summary, the available data do not rule out the possibility that the theory of multiple simultaneous inhibition of dihydrofolate reductase accounts for at least some of the trimethoprim-sulfamethoxazole synergism observed in clinical situations.

MARTIN POE
Merck Institute for Therapeutic Research, Rahway, New Jersey 07065

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Marihuana and Epilepsy

In a recent paper on cannabinoid induction of behavioral seizures in a strain of rabbits (*1*) Martin and Consroe make several statements that should be clarified.

First, they state that in other species electroencephalographic (EEG) patterns of convulsive-like activity and behavioral seizures have only been reported after administration of "lethal or near-lethal doses" of cannabinoids. If accurate, this would suggest that their observations of seizures are perhaps an idiosyncratic strain-specific reponse of their rabbits to cannabinoids, and the relevance to human epilepsy would be unclear. Since they do not report the lethal dose for their strain of rabbits, it is also possible that their epileptogenic dosages were close to the lethal dose (they note the death of one subject). However, we have reported similar results with comparable doses of cannabinoids in two other species; the naturally epileptic beagle dog, and cats with focal epilepsy induced by injection of alumina cream into the motor cortex. In our work (*2*) we have noted activation of temporal lobe seizures and myoclonus in dogs given a single oral dose (5 mg/kg) of Δ^9-tetrahydrocannabinol (Δ^9-THC), a psychoactive ingredient of marihuana. This is not a "near-lethal" dose since we did not observe any fatalities with dosages as high as 20 mg/kg (oral). In the cat we have reported activation of interictal spike discharges in

the EEG from previously quiescent epileptic foci by Δ^9-THC dosages of 1.5 mg/kg (oral). Similar to the results of Martin and Consroe in the rabbit, in neither species did we observe induction of epileptic activity by cannabidiol. Paradoxically, there is a case report (*3*) of increased epileptiform EEG activity in a human epileptic patient after the administration of a high intravenous dose of cannabidiol. While the dosages used in all of these studies are very high compared to the amount taken voluntarily by man (*4*), the pattern of results suggest that in diverse species with differing types of seizures some of the ingredients of marihuana can activate existing epileptic pathology. This may be of some importance, because 29 percent of patients under age 30 use marihuana after being diagnosed epileptic, and this topic of illegal drug use is rarely discussed with their physicians (*5*). Given the findings outlined above, epileptic patients should be at least counseled about possible adverse effects of marihuana use.

A second point that needs comment regards the mechanism by which cannabinoids induce epileptic activity. Martin and Consroe attribute the effect to the "stimulant action of cannabinoids." This is not supported by their own data since the stimulant drug methamphetamine did not produce convulsions in their subjects even at high dosages. Also, the stimulant drug dextroamphetamine is recommended as an anticonvulsant for some types of epilepsy (*6*), and general arousal produced by brain stimulation can arrest focal epileptiform EEG activity (*7*). The mechanism by which cannabinoids precipitate epileptic symptoms may be related to their anticholinergic actions (*8*).

DENNIS M. FEENEY

Department of Psychology, University of New Mexico, Albuquerque 87131

References and Notes

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4. Extrapolation of drug response across diverse species is difficult; however, it should be noted that the epileptogenic dosages used in the animal studies are approximately five times greater than the highest oral and intravenous dosage reported in experimental studies in man and considerably higher than the estimated dose voluntarily taken by man through smoking [L. E. Hollister, *Science* **172**, 21 (19 → M. Perez-Reyes, M. C. Timmons, M. A. Lipton, K. H. Davis, M. E. Wall, *ibid.* **177**, 633 (1972)].
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We emphasize that our findings (*1*) in rabbits of cannabinoid-induced nonfatal behavioral seizures appear novel, and perhaps idiosyncratic, in view of previous reports. Cannabinoids, especially Δ^9-tetrahydrocannabinol (Δ^9-THC) given to rodents, dogs, cats, monkeys, and rabbits over a wide range of doses, cause electroencephalographic (EEG) changes (often interpreted as convulsive-like) without the concomitant appearance of behavioral seizures [see (*2*)]. As Feeney points out, activation or enhancement of EEG seizures by Δ^9-THC in cats (*3*) and by cannabidiol (CBD) in man (*4*) has been reported. However, the relations of all the above EEG effects to behavior are not clear in view of the reported lack of behavioral seizures and in view of additional findings that Δ^9-THC, CBD, and other cannabinoids possess well known, dose-response anticonvulsant effects across laboratory animal species (*2*). Earlier reports of behavioral seizures in rats, dogs and monkeys involved the administration of extremely high (often lethal) doses of Δ^9-THC (*2*). Exceptions to the latter findings are the behavioral convulsions in our rabbits and the myoclonus (and EEG spiking) in Feeney's epileptic beagle dogs (*3*) elicited by Δ^9-THC; we suggest that both these effects are novel and are probably strain-specific or at least strain-sensitive responses. However, while the epileptic beagle dog shows "spontaneous" behavioral convulsions, no "spontaneous" convulsions have ever been observed in our rabbits or, to our knowledge, in any rabbits. Genetically inbred audiogenic rabbits have been described (*5*), but sound, light, and tactile stimuli do not cause seizures in our rabbits (*2*); indeed, the only behavioral convulsions we have observed are those which reliably and immediately follow injection of psychoactive cannabinoids. Furthermore, the median convulsant dose of Δ^9-THC in our rabbits is very low, that is, 0.05 mg/kg, given intravenously (*6*). This dose is far below the median lethal dose, that is, 155 mg/kg, reported for "normal" rabbits (*7*) and below the highest dose we have given to our rabbits, that is, 12 mg/kg, which was not lethal. Indeed, the lowest doses that elicit convulsions in our rabbits are comparable to those producing psychoactive effects in humans. Since the intravenous route of administration produces quantitative effects similar to the inhalational route for active marihuana ingredients (*8*), and the mean inhalation dose for the human is 0.1 mg/kg (*9*), our threshold dose of 0.05 mg/kg is lower than an average human dose. When body surface area of the two species is taken into account, this difference is even larger. The 5.0 mg/kg oral dose of Δ^9-THC which produces seizures in epileptic dogs (*3*) is higher than the average human dose even when one takes into account the fact that an oral dose of Δ^9-THC is about one-third as active as an equivalent intravenous dose.

The one death that we reported (*1*) was due, not to an injection of Δ^9-THC as stated by Feeney, but to an injection of cannabicyclol, a substance which does not go into solution and which does not easily stay in suspension. Rather than being a lethal dose, it is more likely that particles precipitating from the suspension produced pulmonary hemorrhage, as suggested by our autopsy report. The other rabbit who received an injection of cannabicyclol showed no abnormal behavior.

Feeney's second point is that behavioral convulsions are not due to the stimulant action of cannabinoids since the stimulant drug methamphetamine does not produce convulsions. This reasoning assumes that the cannabinoids and methamphetamines have the same mechanism of action; however, there are not enough data to warrant such speculation.

While it is difficult to extrapolate data across species, it is possible that marihuana could affect seizure activity in epileptic patients, as well as interact with clinically used antiepileptic drugs (*10*). Therefore, we fully agree that epileptic patients should be informed of the possible effects of marihuana use.

PARTHENA MARTIN
PAUL CONSROE

Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson 85721

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