Kinetics and Mechanisms of Action of Drugs on Microorganisms VII

Quantitative Adherence of Sulfonamide Action on Microbial Growth to a Receptor-Site Model

By EDWARD R. GARRETT and OLGA K. WRIGHT

The lag time between sulfonamide addition to a balanced growth culture of *Escherichia coli* B/r and the observation of action on microbial growth is 5.5 ± 0.5 generations. It is independent of temperature and the degree of growth inhibition by simultaneously added chloramphenicol. The apparent first-order growth rate constants of *E. coli* in the subsequent drug-affected steady state can be rigorously defined as $k_{app.} = k_0 - k_0K_1K_2S/(1 + K_1K_2S)$ where k_0 is the rate constant in the absence of a sulfonamide concentration, S; K_1 is the steady-state equilibration of sulfonamide between the nutrient medium and the biophase; K_2 is the drug-receptor site association constant; and k_0 and K_1K_2 have been defined as functions of absolute temperature. The death rate of completely sulfonamide-inhibited organisms is independent of sulfonamide concentrations. The ΔH values of $1/K_1K_2$ differ for sulfisoxazole (15.9 Kcal./mole), sulfathiazole (4.3 Kcal./mole), and from the k_0 (24 Kcal./mole). The techniques provide sensitive and reliable estimates of differences in sulfonamide activities. The derived $1/K_1K_2$ values for sulfisoxazole are directly proportional to the growth rate constant in its absence but in the presence of rate-inhibiting chloramphenicol. This indicates that the numbers of receptor sites available for sulfonamide action are directly proportional to the microbial growth rate. The apparent first-order growth rate constant of *E. coli* in the presence of simultaneously added sulfonamide (S) and chloramphenicol (C) concentrations can be rigorously defined as $k_{app.} = [k_0' - k_cC][1 + K_1K_2(S - 1)]/(1 + K_1K_2S)$, where k_0' is the growth rate constant in the absence of chloramphenicol and sulfonamide and k_c is the determined inhibitory constant for chloramphenicol. Variations of sulfonamide concentrations after the lag phase demonstrate that reversibility exists and the ultimate $k_{app.}$ is in accordance with the cited predictive equation. Dilution of organisms but maintenance of sulfonamide concentrations maintains the steady-state growth rate. Addition or dilution of sulfonamide concentrations show a time-dependent re-equilibration. These facts are consistent with a competitive receptor site model for sulfonamide and a generation rate-dependent metabolite which produces modified metabolites that are stored during drug-free balanced growth.

THE VARIATION of the time-course of drug distribution in a complex organism when considered with the concomitant time-course of pharmacological activity can give insight into the properties and nature of the biophase in contact with the drug receptors. Operational models of the complex biological organism are needed to correlate drug–receptor interaction and availability at sites of action. Multicompartmental models can be established to consider and quantitatively define the kinetics of drug absorption, distribution, and biological effectiveness (1). If dosage regimens are varied, deduced compartmental capacities and rate-limiting parameters of the drug distributive processes that result in observed biological activities may functionally define the transport processes and the affinity constants of drug–receptor interaction. Unfortunately, valid quantitative and continuous measurements of pharmacological activity in a complex organism are difficult to obtain.

This paper describes the pharmacokinetic approach to the study of the action of sulfonamides on microbial growth rates in a highly reliable and reproducible system. The kinetic dependencies will be defined as functions of sulfonamide concentrations, variations in such concentrations, temperatures, and numbers of organisms, and will be related to receptor site concepts. Similar studies have been given previously with respect to the action of the chloramphenicols and the tetracyclines on microbial growth rates (2–8).

Kohn and Harris (9, 10) initiated some studies of a kinetic nature on sulfonamide action on microbial growth and made many astute observations from technologically limited data. The variability of pH observed was undesirable since sulfonamide transport and microbial growth rates are claimed to be functions of pH (11, 12).

Received April 6, 1967, from the College of Pharmacy, University of Florida, Gainesville, FL 32601
Accepted for publication August 3, 1967.
Presented in part to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.
The technical assistance of George L. Perry and rewarding discussions with Professor Arthur L. Koch are acknowledged.

It is not clear from their cited evidence (9, 10) if the inoculum was in balanced growth (13, 14) so that the organisms would be statistically distributed among the phases of the growth cycle. If this were not so, it is difficult to dissociate sulfonamide action on microbial growth from accelerating rates of microbial growth in the transition from the induction period to the logarithmic growth phase (2). A rigorous rate constant dependence on sulfonamide concentration was not demonstrated (9, 10).

Modern technological advances with the Coulter counter permit rigorous kinetic analyses of large amounts of data of high reliability and the obtaining of numbers of total intact organisms, live and dead. These can be compared with the numbers of viable organisms obtained by colony count techniques. Coincidence of such numbers of total and viable counts during antibacterial action clearly defines inhibition (5–8).

MATERIALS AND METHODS

The *Escherichia coli* B/r strain was used as the test organism throughout the investigation. The sodium sulfathiazole (Nutritional Biochemicals Corp., Cleveland, Ohio) and sulfisoxazole (Hoffmann-LaRoche Laboratories, Nutley, N. J.) were the drugs studied.

Media—The culture media (15) consisted of 7.00 Gm. K_2HPO_4, 3.00 Gm. KH_2PO_4, 2.0 Gm. glucose, 1.0 Gm. $(NH_4)_2SO_4$, 0.50 Gm. sodium citrate, 0.10 Gm. $MgSO_4 \cdot 7H_2O$, 10.0 Gm. vitamin-free casamino acids (Difco Laboratories, Detroit, Mich.), and 1 L. of distilled deionized water. This medium was filtered twice through a 0.45-mm. Millipore filter and autoclaved at 15 lb. of pressure for 20 min. The pH was 6.90 and did not change throughout the studies. This medium was optimum for the studies in that the time for each study was minimized and the pH maintained constancy throughout the measured growth range. When an inorganic salt–glucose medium was used, the rates of microbial growth were too slow for convenient kinetic studies.

Growth—Replicate slants were prepared from a single colony of *E. coli*, strain B/r, that was isolated from a culture on an agar plate. These slants were frozen and a separate one was used for each experiment. A broth culture was allowed to grow at 37.5° for 16 hr. A sample was then diluted tenfold in fresh broth and the growth was followed by the Coulter counter, model B, (Coulter Electronics Co., Hialeah, Fla.) until it reached an organism concentration of 1×10^8 organisms/ml. in the logarithmic growth phase. Samples were diluted into 5 or 6 replicate 50-ml. volumes of fresh broth in 125-ml. loosely-capped conical flasks to achieve the desired organism concentration. The replicate cultures were maintained at a constant temperature in a 50-gal. constant-temperature water bath equipped with a shaker.

Total Count Method—Appropriate small volume aliquots of each culture were transferred at 40-min. intervals to sterilized vials containing 1 drop of formaldehyde to kill the organisms (5). The samples were appropriately diluted with properly filtered and sterilized 0.9% saline solution so that the total organism count per 50 μl. would not exceed 35,000. The coincidence error was thus less than 0.5% on the Coulter counter model B equipped with a 30-μ orifice. The mean of 3 total counts was obtained on each sample (5–7).

The Coulter counter with this orifice size is able to count particles above 0.5 μ in diameter without significant interference from background noise (Coulter Counter Manual, model B, 4th ed., August 1966). There are negligible numbers of *E. coli* with a volume less than 0.5 μ^3 (14) or a diameter less than 1 μ (16) at our generation rates. Operational counting of *E. coli* has been clearly shown to be effective (5, 14) and in agreement with colony counts. This was also true in the many instances where only bacterial growth inhibition was manifested (5–8).

Viable Count Method—The growing culture, 0.5 ml., was diluted appropriately with 0.9% saline so that 60 to 100 colonies per plate would result. One milliliter of this solution was pipeted onto each of 5 replicate agar plates. The plates were incubated for 48 hr. at 37.5° and the colonies were counted (3–5). Bacto antibiotic medium 3 (Difco Laboratories, Detroit, Mich.) was used for broth cultures and plate counts.

Effect of Drug Concentration on Growth Rates—Six 49-ml. volumes of fresh broth in the constant-temperature baths at 37.5° were inoculated with 1-ml. aliquots of appropriately diluted culture growing in the logarithmic growth phase. The organism population was 10^4 organisms/ml. The cultures were allowed to grow for 80 min. to a population of 10^5 organism/ml. At 80 min., 0.5 ml. of appropriately diluted drug was added to the cultures to produce final concentrations of 1.0, 2.0, 3.0, 4.0, 5.0, and 20.0, 50.0, 75.0, and 100.0 mcg./ml. Samples

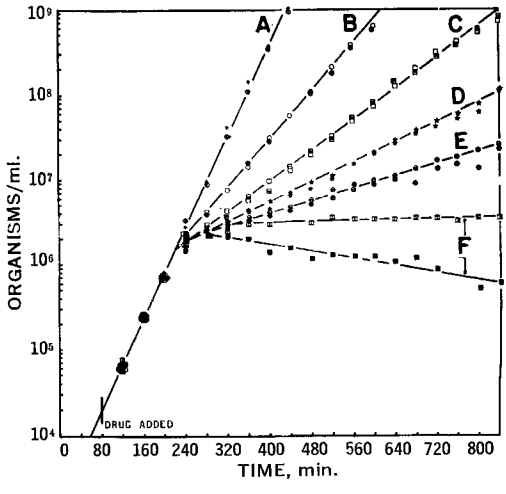

Fig. 1—Typical generation rate curves of E. coli *B/r at 37.5° in the presence of various concentrations of sulfisoxazole. The curves and mcg./ml. concentrations of sulfisoxazole were, respectively:* A, *0.0;* B, *1.0;* C, *2.0;* D, *3.0;* E, *4.0;* F, *20.0. Both viable and total counts were coincident except for the curves* F *where the viable counts (lower curve,* F*) diverged from the total counts (upper curve,* F*).*

were taken every 40 min. and were counted by the Coulter counter method as well as by the method of colony counts (Fig. 1). Sulfisoxazole was tested by both methods. Sodium sulfathiazole was tested only by total counts. A control culture without added drug was also studied to determine the growth rate in the absence of drug.

Effect of Organism Population on Drug-Affected Growth Rates—A series of experiments was conducted at 37.5° in order to check the effect of organism population at the time of drug addition on the bacteriostatic action of sodium sulfathiazole. For each experiment 6 replicate 49-ml. volumes of broth were inoculated with 1 ml. of appropriately diluted culture growing in its logarithmic phase. Experiments were run where the zero time populations were 10^2, 10^3, 10^4, and 5×10^4 organisms/ml. At 80 min. after inoculation, sodium sulfathiazole (0.5 ml. of appropriately diluted master solution) was added to 50 ml. of the cultures to achieve final concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 mcg./ml. One of the 6 replicates had no drug. The organism populations at the time of drug addition were, 10^3, 10^4, 10^5, and 5×10^5 organisms/ml. Samples were taken every 40 min. and the total count per milliliter was obtained on the Coulter counter.

Reversibility of Drug Action—A 100-ml. volume of broth in a 125-ml. conical flask was inoculated with *E. coli* to a concentration of 10^3 organisms/ml. (curve A in Fig. 2). The culture grew logarithmically at 37.5° for 80 min. to a population of about 10^4 organisms/ml. For this and the subsequent variations unless specified otherwise, samples were analyzed for total Coulter count every 20 min. At 80 min. a 1.0-ml. aliquot of an appropriately diluted solution (in fresh broth) of sulfisoxazole was added to the culture to give 1.0 mcg./ml. (curve B) and 5.0 mcg./ml. (curve C). At 340 min., after the culture of curve B (Fig. 2) had well-settled to a new logarithmic growth, 25 ml. of this culture was transferred to a sterile 125-ml. conical flask containing enough sulfisoxazole to increase the drug concentration to 5 mcg./ml. (curve D). The growth of this new culture (curve D, Fig. 2) was followed by total Coulter counts every 40 min. for 960 min.

At 280 min., when the culture of curve C (Fig. 2) growth curve was emerging from the lag period and had not yet reached the new steady state of logarithmic growth, a 5-ml. aliquot of the culture was diluted with 20 ml. of fresh broth in a 125-ml. flask (curve E, Fig. 2).

At 600 min. two 5-ml. aliquots of the culture treated with 5 mcg./ml. sulfisoxazole at 80 min. (curve C, Fig. 2) were diluted with 20 ml. of fresh broth. The final drug concentration of one of the new cultures (curve F, Fig. 2) was thus 1 mcg./ml. Enough drug was added to the second culture to maintain the 5 mcg./ml. drug concentration (curve G, Fig. 2).

Effect of Temperature on Drug-Affected Growth Rates—The inhibitory actions of sodium sulfathiazole and sulfisoxazole on the population growth of *E. coli* were studied at 24.7°, 26.0°, 29.3°, 32.0°, 34.7°, 37.5°, and 39.5°. Logarithmic-phase cultures at 4 concentrations of each drug of 1.0, 2.0, 3.0, and 4.0 mcg./ml., and one culture without drug, were studied by the Coulter count method at each temperature.

Combined Action of Sulfa Drugs and Chloramphenicol—Replicate 50-ml. *E. coli* cultures in the logarithmic growth phase at 37.5° were treated with all combinations of 3 concentrations of chloramphenicol and 4 concentrations of sulfisoxazole. The chloramphenicol concentrations were 0.33, 0.67, and 1.00 mcg./ml. The sulfisoxazole concentrations were 1.0, 2.0, 3.0, and 4.0 mcg./ml.. The drugs were added 80 min. after inoculation of the cultures in the logarithmic growth phase. The Coulter count method was used.

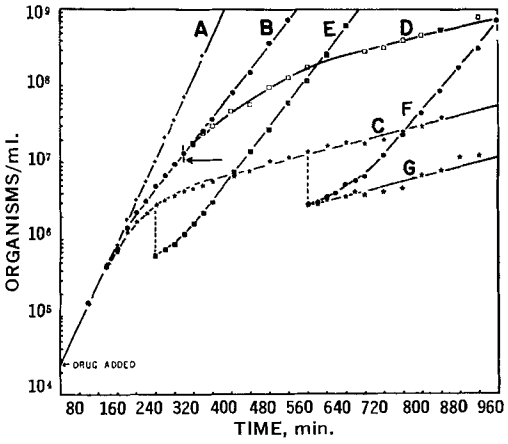

Fig. 2—Generation rate curves of E. coli *B/r at 37.5° on addition and dilution of sulfisoxazole. The population on initial drug addition was 1.6 × 10^4 organisms/ml. The curves, final mcg./ml. concentrations of sulfisoxazole, and apparent steady-state growth rate constants, $k_{app.}$ in sec.$^{-1}$, were:* A, *0.0, 50.2 × 10^{-5};* B, *1.0, 30.2 × 10^{-5};* C, *5.0, 6.29 × 10^{-5};* D, *5.0, 6.00 × 10^{-5};* E, *1.0, 30.9 × 10^{-5};* F, *1.0, 27.6 × 10^{-5};* G, *5.0, 6.28 × 10^{-5}. Arrow on curve* B *denotes more drug added to total 5 mcg./ml.*

RESULTS

Effect of Drug Concentration on Growth Rates—The number of organisms per ml. is plotted against time on semilogarithmic paper for various amounts of sulfa drugs. The example given in Fig. 1 is for sulfisoxazole. A lag period of approximately 120 min. existed before there was any significant effect of any concentration of drug on the population growth of *E. coli* in the logarithmic growth phase at 37.5°. The lag time was the same for both sulfisoxazole and sodium sulfathiazole. Subsequent to the deviation of drug-affected population growth from the control curve, a new linearity in logarithmic growth was attained. At a dosage of 20.0 mcg./ml. that produced complete inhibition of microbial population growth (line F, Fig. 1) and apparent death (line F, Fig. 1) there was an intermediate period of 40–60 min. duration before the new steady-state drug-affected growth curve was established.

At higher dosages of 50.0, 75.0, and 100.0 mcg./ml. the growth curves were superimposable and independent of the dose. Subsequent to the lag period for these higher dosages, the rate of growth decreased, and the maximum value in the number of organisms was obtained 50 min. after the deviation from the initial growth period. A slowly accelerating decay rate in viable organisms was then ob-

TABLE I—EFFECT OF SODIUM SULFATHIAZOLE CONCENTRATIONS, S, ON STEADY-STATE POPULATION GROWTH RATE CONSTANTS, $k_{app.}$ IN SEC.$^{-1}$ FOR VARIOUS POPULATION SIZES OF *E. coli.*, *N*, AT TIME OF ADDITION AT 37.5°

S, mcg./ml.	*N*: 1 × 10³	1.2 × 10⁴	1 × 10⁵	6 × 10⁵
	$k_{app.}$			
0.0	$k_0 = 45.7 \times 10^{-5}$	49.1×10^{-5}	48.0×10^{-5}	48.7×10^{-5}
0.5	36.1×10^{-5}	...	...	...
1.0	...	27.7×10^{-5}	29.2×10^{-5}	29.3×10^{-5}
2.0	18.0×10^{-5}	16.5×10^{-5}	23.5×10^{-5}	21.6×10^{-5}
3.0	10.4×10^{-5}	9.9×10^{-5}	14.9×10^{-5}	12.4×10^{-5}
4.0	7.1×10^{-5}	7.0×10^{-5}	9.7×10^{-5}	10.8×10^{-5}
5.0	4.6×10^{-5}	4.4×10^{-5}	6.2×10^{-5}	...
k_b/k_a[a]	2.3×10^3	1.9×10^3	1.7×10^3	1.8×10^3
$1/k_a$[a]	3.4×10^3	3.1×10^3	3.6×10^3	3.4×10^3

[a] These values were obtained from the slope and intercept of the plot $S/(k_0 - k_{app.})$ *versus* S in accordance with the expression $S/(k_0 - k_{app.}) = 1/k_a + (k_b/k_a)\,S$.

TABLE II—EFFECT OF SULFISOXAZOLE CONCENTRATION, S, ON STEADY-STATE POPULATION GROWTH RATE CONSTANTS, $k_{app.}$ IN SEC.$^{-1}$, FOR SEVERAL TEMPERATURES[a]

S, mcg./ml.	Temp.: 24.7°C.	26.0°C.	29.3°C.	32.0°C.	34.7°C.	37.5°C.	39.5°C.
				$k_{app.}$			
0.0	$k_0 = 2.13 \times 10^{-4}$	2.34×10^{-4}	3.18×10^{-4}	3.99×10^{-4}	4.28×10^{-4}	5.03×10^{-4}	5.31×10^{-4}
1.0	8.00×10^{-5}	9.02×10^{-5}	1.40×10^{-4}	2.09×10^{-4}	2.56×10^{-4}	3.12×10^{-4}	3.33×10^{-4}
2.0	3.79×10^{-5}	4.67×10^{-5}	7.08×10^{-5}	1.16×10^{-4}	1.75×10^{-4}	1.74×10^{-4}	2.22×10^{-4}
3.0	2.68×10^{-5}	2.26×10^{-5}	4.87×10^{-5}	8.05×10^{-5}	1.34×10^{-4}	1.44×10^{-4}	1.59×10^{-4}
4.0	1.53×10^{-5}	1.38×10^{-5}	2.99×10^{-5}	6.40×10^{-5}	9.00×10^{-5}	1.08×10^{-4}	1.10×10^{-4}
k_b/k_a[b]	4.23×10^3	3.71×10^3	2.97×10^3	2.27×10^3	2.04×10^3	1.73×10^3	1.39×10^3
$1/k_a$[b]	2.99×10^3	3.21×10^3	3.43×10^3	3.10×10^3	3.72×10^3	3.47×10^3	3.92×10^3
$k_a/k_b = qk_m$[c]	2.37×10^{-4}	2.70×10^{-4}	3.37×10^{-4}	4.41×10^{-4}	4.91×10^{-4}	5.77×10^{-4}	7.18×10^{-4}
$k_b = K_1K_2$[d]	1.42	1.16	0.865	0.780	0.550	0.499	0.355

[a] Sulfisoxazole added to 10^5 organisms/ml. [b] These values were obtained from the slope and intercept of the plot $S/(k_0 - k_{app.})$ *versus* S in accordance with the expression $S/(k_0 - k_{app.}) = 1/k_a + (k_b/k_a)\,S$. [c] Reciprocal of slope of such a plot. [d] Ratio of slope to intercept of such a plot.

TABLE III—EFFECT OF SODIUM SULFATHIAZOLE CONCENTRATION, S, ON STEADY-STATE POPULATION GROWTH RATE CONSTANTS, $k_{app.}$ IN SEC.$^{-1}$, FOR SEVERAL TEMPERATURES[a]

S, mcg./ml.	Temp.: 24.7°C.	26.0°C.	29.3°C.	32.0°C.[b]	34.7°C.	37.5°C.	39.5°C.
				$k_{app.}$			
0.0	$k_0 = 2.13 \times 10^{-4}$	2.35×10^{-4}	3.14×10^{-4}	3.95×10^{-4}	4.36×10^{-4}	4.76×10^{-4}	5.31×10^{-4}
1.0	1.18×10^{-4}	1.24×10^{-4}	1.65×10^{-4}	2.15×10^{-4}	2.67×10^{-4}	2.92×10^{-4}	3.25×10^{-4}
2.0	6.80×10^{-5}	8.54×10^{-5}	1.11×10^{-4}	1.49×10^{-4}	2.04×10^{-4}	2.35×10^{-4}	2.27×10^{-4}
3.0	3.67×10^{-5}	4.94×10^{-5}	6.95×10^{-5}	1.05×10^{-4}	1.60×10^{-4}	1.49×10^{-4}	1.70×10^{-4}
4.0	3.15×10^{-5}	2.98×10^{-5}	5.47×10^{-5}	8.43×10^{-5}	7.85×10^{-5}	9.71×10^{-5}	1.28×10^{-4}
k_b/k_a[c]	3.77×10^3	3.41×10^3	2.85×10^3	2.21×10^3	1.90×10^3	1.66×10^3	1.50×10^3
$1/k_a$[c]	5.96×10^3	5.46×10^3	5.23×10^3	4.04×10^3	4.07×10^3	3.56×10^3	3.16×10^3
$k_a/k_b = qk_m$[d]	2.66×10^{-4}	2.93×10^{-4}	3.51×10^{-4}	4.53×10^{-4}	5.27×10^{-4}	6.02×10^{-4}	6.67×10^{-4}
$k_b = K_1K_2$[e]	0.632	0.626	0.544	0.547	0.466	0.466	0.476

[a] Sodium sulfathiazole added to 10^5 organisms/ml. [b] $k_{app.} = 6.22 \times 10^{-5}$ sec.$^{-1}$ at 5.0 mcg./ml. of sodium sulfathiazole. [c] These values were obtained from the slope and intercept of the plot $S/(k_0 - k_{app.})$ *versus* S in accordance with the expression $S/(k_0 - k_{app.}) = 1/k_a + (k_b/k_a)\,S$. [d] Reciprocal of slope of such a plot. [e] Ratio of slope to intercept of such a plot.

TABLE IV—EFFECT OF SULFISOXAZOLE CONCENTRATION, S, ON STEADY-STATE POPULATION GROWTH RATE CONSTANTS, $k_{app.}$ IN SEC.$^{-1}$, FOR SEVERAL CHLORAMPHENICOL CONCENTRATIONS, C, AT 37.5°C.[a]

S, mcg./ml.	C, mcg./ml.[b]: 0.33	0.67	1.00
		$k_{app.}$	
0.00	$k_0 = 4.08 \times 10^{-4}$	3.41×10^{-4}	2.38×10^{-4}
1.00	2.44×10^{-4}	2.07×10^{-4}	1.22×10^{-4}
2.00	1.63×10^{-4}	1.45×10^{-4}	0.95×10^{-4}
3.00	1.02×10^{-4}	1.13×10^{-4}	0.58×10^{-4}
4.00	0.86×10^{-4}	0.85×10^{-4}	0.47×10^{-4}
k_b/k_a[c]	2.23×10^3	2.96×10^3	4.22×10^3
$1/k_a$[c]	4.10×10^3	4.40×10^3	4.40×10^3
$k_a/k_b = qk_m$[d]	4.48×10^{-4}	3.38×10^{-4}	2.37×10^{-4}
$k_b = K_1K_2$[e]	0.544	0.673	0.960
$1/k_b$	1.84	1.49	1.04

[a] Sulfisoxazole and chloramphenicol added to 10^5 organisms/ml. [b] The $k_{app.}$ values in sec.$^{-1}$ prior to the addition of chloramphenicol 0.33, 0.67, and 1.00 mcg./ml. were 5.10, 5.12, and 5.17 × 10^{-4}, respectively. [c] These values were obtained from the slope and intercept of the plot $S/(k_0 - k_{app.})$ *versus* S in accordance with the expression: $S/(k_0 - k_{app.}) = 1/k_a + (k_b/k_a)\,S$. [d] Reciprocal of slope of such a plot. [e] Ratio of slope to intercept of such a plot.

served for 120 min. Subsequently, the logarithmic decay of the numbers of viable organisms was constant with time for these doses with an apparent first-order rate constant of 9.75×10^{-5} sec.$^{-1}$.

The total count technique showed a decreasing rate of increase in total numbers over 120 min. after the deviation from the initial growth curve. At that time and for 400 subsequent min., there was a negligible change in the total number of organisms.

Apparent population growth rate constants, $k_{app.}$ in sec.$^{-1}$, were obtained from the slopes of these new linear logarithmic growth curves in accordance with the apparent first-order expression:

$$\log N = (k_{app.}/2.303)t + \text{constant} \quad \text{(Eq. 1)}$$

where t is time and N is the number of organisms/ml. The $k_{app.}$ values for various concentrations of sodium sulfathiazole and sulfisoxazole under varied conditions are given in Tables I–IV.

When the $k_{app.}$ values are plotted against the concentrations of the sulfa drugs, the curves are nonlinear and demonstrate that the $k_{app.}$ is not a linear function of the concentrations of the sulfa drugs. However, when the reciprocal of the differences between the population growth rate constant in the absence of drug, k_0, and the $k_{app.}$ values for population growth in the presence of drug are plotted against the reciprocal of the concentration of the sulfa drug, S in mcg./ml., excellent linearity is obtained (Fig. 3 for sulfisoxazole) in accordance with the expression:

$$1/(k_0 - k_{app.}) = (1/k_a)\,(1/S) + k_b/k_a \quad \text{(Eq. 2)}$$

where k_a and k_b are constants.

Similarly, when Eq. 2 is multiplied through by the concentration of the sulfa drug, this concentration divided by the difference between the population growth rate constants in the presence and absence of sulfa drug is a linear function of sulfa drug concentration, S, as in Fig. 4 for sulfathiazole:

$$S/(k_0 - k_{app.}) = 1/k_a + (k_b/k_a)S \quad \text{(Eq. 3)}$$

The intercepts, $1/k_a$, and slopes, k_b/k_a, derived from such plots are also given in Tables I–IV.

Effect of Temperature on Drug-Affected Growth Rates—The data for the effect of sulfonamide drugs on the steady-state growth of *E. coli* (Tables II and III) were analyzed by application of Eqs. 2 and 3 for each temperature studied, and the parameters k_a/k_b and k_b were determined from the slopes and intercepts of the plots in accordance with these equations (Figs. 3 and 4). The k_a and k_b parameters reasonably fit the Arrhenius or Van't Hoff equation for the dependence of reaction rate or equilibrium constants upon temperature (Fig. 5):

$$\log k = \log P - (\Delta H/2.303\,R)\,(1/T) \quad \text{(Eq. 4)}$$

where R is 1.987 Kcal./mole and T is in degrees Kelvin. The apparent ΔH values for k_b are -15.9 Kcal./mole for sulfisoxazole and -4.3 Kcal./mole for sodium sulfathiazole. For both sulfonamide studies, the apparent heat of activation for the derived k_a/k_b was 24 Kcal./mole. Similar plots for the k_0 values, *i.e.*, the population growth rate constants at the various temperatures in the absence of any sulfonamide were parallel to the plots for the k_a/k_b values and thus of the same apparent ΔH values.

The interval from the time of addition of sulfonamide to the time when the population growth rate

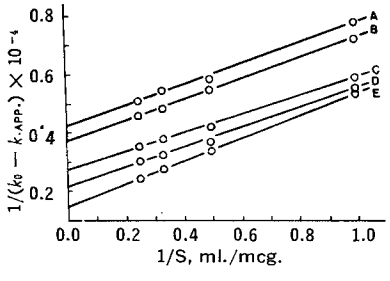

Fig. 3—Examples of quantitative relations between apparent E. coli *growth rate constants,* $k_{app.}$, *and sulfisoxazole concentrations,* S, *at various temperatures. The curves are plotted in accordance with the expression* $1/(k_0 - k_{app.}) = (1/k_a)(1/S) + k_b/k_a$ *where the growth rate constant in the absence of sulfonamide,* k_0, *and* $k_{app.}$ *are in sec.*$^{-1}$ *and S is in mcg./ml. The curves and respective temperatures are:* A, *24.7°;* B, *26.0°;* C, *29.3°;* D, *32.0°;* E, *39.5°.*

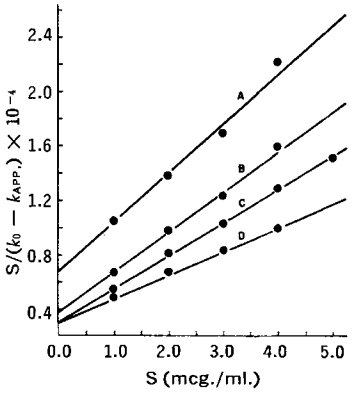

Fig. 4—Examples of quantitative relations between apparent E. coli *growth rate constants,* $k_{app.}$, *and sodium sulfathiazole concentrations, S, at various temperatures. The curves are plotted in accordance with the expression* $S/(k_0 - k_{app.}) = 1/k_a + (k_b/k_a)S$ *where the growth rate constant in the absence of sulfonamide,* k_0, *and* $k_{app.}$ *are in sec.*$^{-1}$ *and S is in mcg./ml. The curves and respective temperatures are:* A, *24.7°;* B, *29.3°;* C, *32.0°;* E, *39.5°.*

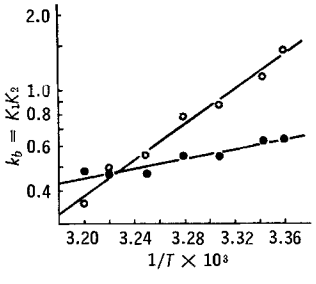

Fig. 5—The variation of a derived parameter, $k_b = K_1K_2$, *of sulfonamide action on* E. coli *microbial growth as a function of temperature. The curves for sulfisoxazole* (○) *and sulfathiazole* (●) *are plotted in accordance with the expression:* $\log k_b = -\Delta H/2.3RT + \log P$ *where T is the absolute temperature.*

demonstrates a deviation to a drug-affected rate is termed the lag time (Fig. 1). This lag time is inversely proportional to the growth rate constant in the absence of sulfonamides for all temperatures studied. The linearity of the plot of lag time *versus* $1/k_0$ is demonstrated in Fig. 6. At any temperature, this lag time corresponded to 5.5 ± 0.5 generations.

Effect of Chloramphenicol on Sulfonamide-Affected Growth Rate—Chloramphenicol and sulfisoxazole were added simultaneously to a growing culture of *E. coli* at 10^6/ml. The chloramphenicol caused an almost instantaneous change in the rate of growth, but the sulfonamide effect was not observed until after a definitive lag time. Subsequent to this lag time, a drug-equilibrated growth rate was observed which was an apparent function of sulfisoxazole concentration. A typical semilogarithmic plot of such growth curves is given in Fig. 7 for 0.666 mcg./ml. chloramphenicol and 0.0 to 4.0 mcg./ml. sulfisoxazole.

The lag time from the time of addition of both drugs to the time when sulfonamide action was demonstrated was inversely proportional to the growth rate constant in the absence of sulfonamide (Fig. 6). At any chloramphenicol concentration used this lag time corresponded to 5.5 ± 0.5 generations.

The data for the effect of combined sulfonamide and chloramphenicol drugs on the steady-state growth rate constants for *E. coli* after the lag time are given in Table IV. These data were consistent with Eqs. 2 and 3 as demonstrated in the plot given in Fig. 8. The k_0' values chosen were those values in the presence of chloramphenicol but in the absence of sulfisoxazole. The slopes and intercepts of the curves are also given in Table IV.

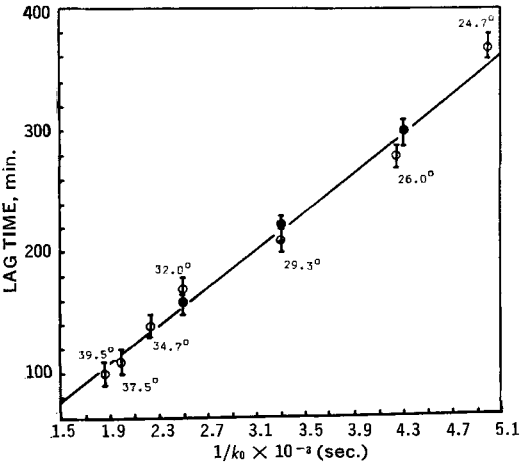

Fig. 6—Lag-time before sulfonamide affects E. coli *growth as a function of the generation time, $1/k_0$, at the time of sulfonamide addition. Key: ○, estimated lag times for sulfathiazole and sulfisoxazole-affected microbial cultures at the various cited temperatures; ●, lag times for microbial cultures simultaneously treated with sulfonamide and various concentrations of chloramphenicol at 37.5°. The vertical lines through the points represent ranges of the estimated values. The k_0 values are the observed growth rate constants in sec.$^{-1}$.*

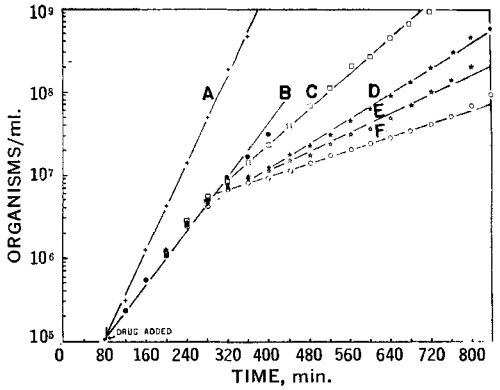

Fig. 7—Typical generation rate curves of E. coli *B/r at 37.5° in the presence of chloramphenicol and various concentrations of sulfisoxazole. The population on the addition of both drugs was 10^5 organisms/ml. Curve* A *is for the growth rate in the absence of both drugs and curve* B *is for 0.666 mcg./ml. of chloramphenicol. The other curves were for 0.666 mcg./ml. of chloramphenicol and the following mcg./ml. of sulfisoxazole:* B, *0.0;* C, *1.0;* D, *2.0;* E, *3.0;* F, *4.0.*

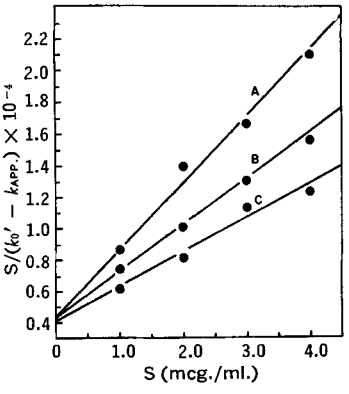

Fig. 8—Quantitative relations between apparent E. coli *growth rate constants, $k_{app.}$, and sulfisoxazole concentrations, S, at various chloramphenicol concentrations at 37.5°. The curves are plotted in accordance with the expression $S/(k'_0 - k_{app.}) = 1/k_a + (k_b/k_a)S$ where the growth rate constant in the absence of chloramphenicol and sulfisoxazole, k_0, is related to the growth rate constant, k_0', in the presence of chloramphenicol by $k_0' = k_0 + k_cC$ where C is the concentration of chloramphenicol in mcg./ml. and $k_c = 2.68 \times 10^{-4}$ ml./mcg./sec. The curves and respective concentrations in mcg./ml. of chloramphenicol are:* A, *1.00;* B, *0.67;* C, *0.33.*

Effect of Organism Population on Drug-Affected Growth Rates—The apparent growth rate constants for *E. coli*, $k_{app.}$, obtained in the presence of various concentrations of sodium sulfathiazole at various inoculum sizes at time of drug addition are listed in Table I. The values were obtained from the slopes of the curves of the logarithm of the total organism count *versus* time after the steady state attributed to sulfa drug effects had resulted as demonstrated in Fig. 1. There were no large differences in the $k_{app.}$ values obtained for the various organism population sizes on addition of a given concentration of sodium sulfathiazole for the particular *E. coli* culture used

(Table I). The variations were greatest for higher organism and sulfa drug concentrations. These facts imply that it is best to do comparative studies of sulfonamide action on *E. coli* growth at as constant a population as is possible. For this reason, sulfonamide was added at about 10^6 organisms/ml. Similar studies were also performed with different *E. coli* B/r cultures. In many of these cases, organism population had a greater effect on drug-equilibrated growth rates than with the specific culture used throughout these experiments.

Reversibility of Drug Action—As previously stated, the addition of sulfonamide to an inoculum demonstrated no immediate effect on the microbial growth rate (curves A and B, Fig. 2). It takes 5.5 generations for a drug effect on the microbial growth rate to be observed. At the lower concentrations of drug (curve B, Fig. 2), the transition period to the new steady-state growth rate can be considered as small and is 20–30 min. for 1.0 mcg./ml. of sulfisoxazole. At the higher concentrations of drug (curve C, Fig. 2), the transition period to the new steady-state growth rate is larger and is 140–160 min. for 5.0-mcg./ml.-affected growth rate (curve D). The ultimate steady-state growth rate achieved is independent of whether the total sulfisoxazole is added to the initial culture (curve C), or added stepwise (curves B and D), or the drug-affected culture is diluted and sufficient drug is added to maintain the same concentration (curves C and G). The growth rate constant, $k_{app.}$, was the same in all these instances when steady state was attained.

When the culture in the drug-affected growth period is diluted so that the sulfisoxazole concentration is diminished, the growth rate increases (curve C to E and to F) to achieve a new steady-state rate of growth. The increase in rate appears to occur immediately after dilution, but 100–130 min. must elapse before the new steady-state rate of growth is achieved. The ultimate growth rate is equivalent to that which would have been ultimately achieved with the same concentration of drug added to fresh inoculum.

DISCUSSION

The action of sulfonamide on drug-equilibrated microbial growth in the individual cell may be explained by the simple model:

$$S \underset{K_1}{\overset{}{\rightleftharpoons}} S' + R \underset{K_2}{\overset{k_2}{\rightleftharpoons}} (S'R) \quad \text{(Eq. 5)}$$

This model assumes an equilibrated partitioning of the sulfonamide concentration S in the media with the concentration S′ in the cell which is available to the unreacted receptor sites, R. The fact that the growth rate is constant for a given sulfonamide in the drug-equilibrated steady state implies that the two equilibria characterized by K_1 and K_2 are achieved in that state.

If θ is the fraction of receptor sites reacted with drug, the metabolic rate, $d\,M/dt$, may be proportional to the fraction, $1 - \theta$, of receptor sites that are unreacted with drug:

$$d\,M/dt = k_m\,(1 - \theta) \quad \text{(Eq. 6)}$$

An implicit assumption in this postulate is that the number of receptor sites in a single bacterium are constant and independent of the mass of the cell or time. Possible cases where this was not assumed have been discussed previously (7).

The equilibrium constant, K_2, for the drug–site interaction of Eq. 5 may be defined by:

$$K_2 = k_2/k_1 = (S'R)/[R_t - (S'R)]\,(S') = (S'R)/(S')(R) \quad \text{(Eq. 7)}$$

where $(S'R)$ is the number of sulfonamide-reacted sites, R is the number of unreacted sites, and R_t is the total number of sites whether reacted or unreacted. The fraction θ of sites reacted with drug may be obtained from rearrangement of Eq. 7:

$$\theta = (S'R)/R_t = K_2S'/(1 + K_2S') \quad \text{(Eq. 8)}$$

Thus, Eq. 6 on substitution becomes:

$$d\,M/dt = k_m - k_mK_2S'/(1 + K_2S') \quad \text{(Eq. 9)}$$

where k_m is the steady-state rate of metabolism in the absence of sulfonamide.

The concentration of sulfonamide, S′, in the cell is related to the concentration, S, in the media by:

$$S' = K_1S \quad \text{(Eq. 10)}$$

It follows that the rate of metabolism in the steady state of a single bacterium is related to the sulfonamide concentration, S, in the media by:

$$d\,M/dt = k_m - k_mK_1K_2S/(1 + K_1K_2S) \quad \text{(Eq. 11)}$$

If it is postulated that the rate of population increase of N organisms is proportional to this rate of metabolism and to the number of organisms in the balanced growth culture, it follows that:

$$d\,N/dt = q(dM/dt) \times N \quad \text{(Eq. 12}a\text{)}$$

$$d\,N/dt = [qk_m - qk_mK_1K_2S/(1 + K_1K_2S)]N \quad \text{(Eq. 12}b\text{)}$$

$$d\,N/dt = [k_0 - k_aS/(1 + k_bS)] \times N \quad \text{(Eq. 12}b\text{)}$$

$$d\,N/dt = k_{app.} \times N \quad \text{(Eq. 12}c\text{)}$$

where $k_0 = qk_m = k_a/k_b$, $k_a = qk_mK_1K_2$, $k_b = K_1K_2$ and:

$$k_{app.} = k_0 - k_aS/(1 + k_bS) \quad \text{(Eq. 13)}$$

where $k_{app.}$ is the apparent first-order steady-state growth rate constant in the presence of a constant sulfonamide concentration, S.

On integration, Eq. 12c becomes Eq. 1, and the $k_{app.}$ values were obtained from the slopes of semilogarithmic plots of drug-equilibrated growth (Figs. 1, 2, and 7; Tables I–IV).

The Eq. 13 can be rearranged into the forms of Eqs. 2 and 3, from which by appropriate plotting (Figs. 3, 4, and 8) the k_a and k_b values and related parameters were obtained (Tables I–IV).

The adherence of the data to Eqs. 2 and 3 demonstrates the validity of the model of Eq. 5 to explain the action of sulfonamide on microbial growth. The metabolic rate in the drug-affected steady state that results in microbial growth is proportional to the number of receptor sites unreacted with or unbound by sulfonamide. These receptor sites may be enzymes that are needed in the metabolic pathway (17–22) or an actual and constant amount of

substrate needed in the metabolic sequence that is inactivated by the sulfonamide (23, 24).

It has been argued (17–24) that certain sulfonamide antagonists such as *p*-aminobenzoic acid (PABA) and the sulfonamide compete for such receptor sites, and PABA reaction is necessary in the metabolic sequence. If this is true, the experimental confirmation of Eq. 5 demonstrates that all available receptor sites are occupied either by sulfonamide or PABA and the relative degree of occupation is determined competitively.

It is implied by the experimental verification of Eqs. 5–13 that total reaction of sulfonamide with the total number of receptor sites, R_t, is necessary for complete cessation of microbial growth. This should mean that the obtained $k_a/k_b = gk_m$ (Eqs. 2, 3, 12, and 13) should have the same magnitude as k_0, the apparent growth rate constant in the absence of sulfonamide. This is not exactly true as is evidenced by the values given in Tables II and III. For sulfisoxazole, $k_0 = 0.90\ k_a/k_b$, and for sodium sulfathiazole, $k_0 = 0.82\ k_a/k_b$, with average deviations of 0.03. These facts imply that it is only necessary to affect 82% or 90% of the available receptor sites to obtain the asymptotic cessation of microbial growth by these sulfonamides. The detailed analysis of this concept has been given previously (7).

Although the major action of sulfonamides is inhibitory, microorganism death is observed at high sulfonamide concentrations. Viable and total organism counts are coincident for 0.0 to 4.0 mcg./ml. of sulfisoxazole and demonstrate that microbial growth inhibition is the effective process in this range (Fig. 1). However, at much higher doses of sulfisoxazole, which included 20.0, 50.0, 75.0, and 100.0 mcg./ml., the constancy of the drug-equilibrated total microbial count with time (curve F, Fig. 1) implied completely inhibited growth. The loss of viables by a first-order process of dying must be assigned to the death of growth-inhibited microorganisms. In studies with 50.0, 75.0, and 100.0 mcg./ml., the viable count with time did not vary among these sulfisoxazole concentrations and went through a maximum after the lag period. The same ultimate, first-order kill rate was observed at 37.5° for all these concentrations. These observations imply that the nongrowing, sulfonamide-affected microorganism does not lose its viability as a function of sulfonamide concentration. It is probable that the metabolic limitations placed on the organisms by saturation of receptor sites with sulfonamide results in a statistical probability of death demonstrated by a first-order rate of dying.

It has been shown previously (7) that a plot of the logarithm of the microbial growth rate constant against the reciprocal of the absolute temperature is reasonably linear for drug-free *E. coli* cultures in the temperature range 25° to 39° and that ΔH values can be determined from the slopes of such plots in accordance with Eq. 4. This reasonable adherence to the Arrhenius or Van't Hoff Eq. 4 implies that one step, or several steps with similar temperature dependencies, in the metabolic processes of the organism may be rate limiting in this temperature range. The coincidence of the apparent ΔH values, 24 Kcal./mole for the derived k_a/k_b values from the sulfonamide studies (Tables II and III) and the observed k_0 values in the absence of sulfonamide indicate that these constants represent the same processes.

The derived parameter most descriptive of the quantitative action of a sulfonamide on microbial growth is $k_b = K_1K_2$ (Tables II–IV). Unfortunately, this product cannot be kinetically dissociated into the partition coefficient or transfer process for drug across the cell membrane, K_1, and the affinity constant, K_2, for sulfonamide reaction with receptor site. The product of these two equilibrium constants is temperature dependent (Fig. 5) and is reasonably in accordance with the Arrhenius or Van't Hoff Eq. 4 over the temperature range studied. The ΔH from this equation is not the same for sulfisoxazole and sulfathiazole. In terms of dissociation constants, $1/K_2$, and partition coefficient for the ratio of concentrations in the media to that within the cell, *i.e.*, $1/K_1$, the ΔH of $1/K_1K_2$ is 15.9 Kcal./mole for sulfisoxazole and 4.3 Kcal./mole for sulfathiazole. These values are significantly different from the ΔH of 24 Kcal./mole for microbial growth in the absence of sulfonamide, and are significantly different from each other. These facts imply that a fundamental difference exists between the reactivities and/or transport of these two sulfonamides into the cell.

Heats of diffusion are generally small, about 2–5 Kcal./mole. Thus, if partition or transport of the sulfonamide into the cell is a diffusion-controlled passive process, large ΔH values would indicate that definitive heats of reaction may be assigned to drug–receptor site interaction since:

$$\ln K_1K_2 = \ln P_{K_1K_2} - \Delta H_{K_1K_2}/RT = \ln K_1 + \ln K_2 = \ln P_{K_1}P_{K_2} - (\Delta H_{K_1} + \Delta H_{K_2})/RT \quad \text{(Eq. 14)}$$

so that:

$$\Delta H_{K_1K_2} = \Delta H_{K_1} + \Delta H_{K_2} \quad \text{(Eq. 15)}$$

For example, the $\Delta H_{K_1K_2}$ values for these two sulfonamides may imply that the receptor site–drug interaction or active transport processes have greater significance in the sulfisoxazole case than in the sulfathiazole case.

Several mechanisms of sulfonamide action have been proposed. One that has had wide acceptance is that these drugs act competitively with essential metabolites such as *p*-aminobenzoic acid, PABA, in a process which is necessary for growth (17–24). This can be competition for an enzyme in a vital enzymatic transformation (17–22), or is actually a competitive chemical reaction involving a vital metabolite in a necessary metabolic sequence (23, 24). To be consistent with our experimentally verified Eqs. 13 and 14, acceptance of these concepts implies that all receptor sites in Eq. 5 (whether enzymatic *or* chemical) are occupied by or reacted with either the sulfonamide or the available PABA in the drug-affected steady state. The number of sulfonamide-unreacted sites or substrates, *i.e.*, R are really PABA-reacted sites or PABA-reacted substrates and the rate of metabolism linked to growth is proportional to this R.

The lag time is the interval from the time of addition of sulfonamide to the time when the population growth rate demonstrates a deviation to a drug-affected rate (9, 25). This lag time is now shown to be inversely proportional to the growth

rate constant before sulfonamide affects the growth rate for all temperatures studied, and when chloramphenicol was added concomitantly with the sulfonamide (Fig. 6). At any chloramphenicol concentration and for any temperature, this lag time corresponded to 5.5 ± 0.5 generations. The 5.5 ± 0.5 generations after sulfonamide addition and before an observed deviation in microbial growth rate imply that cellular division is primarily responsible for the conditions necessary for sulfonamide action or microbial growth to be observed. This could be assigned to the depletion of PABA-derived metabolite [or folic acid precursor (22, 26–28)] storage in a single cell, in agreement with the arguments of Kohn and Harris (9, 10) and Rose and Fox (29). As long as these compounds are in excess, the metabolic pipeline can be maintained full and the rate of microbial growth dependent on a delivered quantity of metabolite per unit of time is invariant. The limiting factor in microbial growth may be the size of this pipeline. In more conventional terms, this would be equivalent to the saturation value of a compartment, tissue, enzyme, or process. It also follows that a standard excess of PABA metabolite and PABA is produced in the balanced growth culture.

Evidence for this is provided by the variation in the dose-response of sulfonamide action on drug-affected steady-state microbial growth as a function of inoculum size. Although this was of little significance in the inoculum ranges with the particular culture used in this paper, many other cultures (30–32), including other strains of *E. coli* B/r that were investigated, showed this effect in varying degrees. In general, for these other strains the greater the number of organisms on drug addition, the more sulfonamide was needed to give the same inhibition of microbial growth, or the greater the antagonism to sulfonamide action. If each organism in a balanced growth culture produced an excess of PABA or PABA-derived metabolites which "leaked" into the nutrient media, the response to sulfonamide would obviously be a function of the numbers of such organisms prior to the inhibition of production of PABA or PABA-derived metabolite.

Ultimately, a situation results where the amounts of stored PABA metabolites per unit cell are insufficient to saturate the pipeline and a gradual decrease in rate of microbial growth occurs to the new drug-affected steady state. This gradual change in rate also may be attributed in part to cellular division and thus to depletion of PABA metabolites in a single cell. The new drug-affected steady state then may be assigned to the competition of PABA from its new steady-state production and the cellular sulfonamide concentration. They compete for all available receptor sites and the production of PABA metabolites depends on the results of this competition.

An alternative or concomitant possibility is the diminution of available receptor sites (or enzymes) in the new steady state (27). Since depletion of stored PABA metabolites explains the lag period (9, 10, 29), lowered rates of PABA production and/or decreased numbers of receptor sites due to the feedback from metabolites' inhibition by sulfonamide are plausible. As first approximations, the rate of PABA production and/or the numbers of available receptor sites may be assumed to be proportional to the microbial growth rate.

The greater the sulfonamide concentration of the balanced growth culture (Fig. 1), the longer the time to achieve the new drug-affected steady state. This may imply time-dependent processes either for equilibration of sulfonamide in the culture media with the biophase concentration, or for diminution of rates of PABA production, or for transfer of PABA into the biophase.

The sulfonamide concentration was reduced by dilution of the culture with nutrient media or was increased by adding more sulfonamide (Fig. 2). This was done after the lag phase, both before and during the drug-affected steady-state growth process. There were gradual transitions to the new steady-state microbial growth rate characteristic of the new sulfonamide concentration. Since PABA-derived metabolites were already depleted, this may be assigned to either one or a combination of the following: (*a*) a time interval is necessary to rejuvenate or inhibit PABA production to its new steady state; (*b*) a finite time interval is necessary to equilibrate receptor sites with sulfonamide and/or PABA; (*c*) the equilibration between sulfonamide in the biophase surrounding the receptor sites and the sulfonamide in the culture media is a time-dependent process; (*d*) the biophase compartment is removed from the PABA generation site and the distributive processes for PABA are time-dependent; (*e*) there is a lag in transfer of the PABA-derived metabolite to or in the metabolic pathway that results in metabolism and growth.

The authors have previously made the statement that, as first approximations, the rate of PABA production and/or the numbers of receptor sites (*e.g.*, folic acid synthesizing enzymes) may be assumed to be proportional to the microbial growth rate. These postulates would explain the appearance of a new drug-affected steady state of microbial growth where the new rate of PABA production and the sulfonamide in the cell would compete for available receptor sites. Thus, the numbers of sulfonamide-unaffected sites, R in Eq. 5, that give rise to metabolism and growth, are PABA-reacted and would be direct functions of PABA production. It follows from Eq. 7 that the sulfonamide–receptor site dissociation constant, $1/K_2$ is directly proportional to this number of receptor sites, R, and thus should be directly proportional to the microbial growth rate. The same reasoning results if a feedback mechanism is operative (27) and the numbers of folic acid synthesizing enzymes are reduced with a reduced microbial growth rate.

Evidence in support of these statements is provided by the studies of microbial growth on simultaneous addition of chloramphenicol and sulfisoxazole (Table IV, Fig. 7). Confirmation of the adherence of the data to Eqs. 2, 3, 12, and 13 is demonstrated by the plots of Fig. 8. The derived parameters are listed in Table IV. It is apparent that the obtained K_1K_2 values, all at 37.5°, vary as a function of the chloramphenicol concentration, C, as do the k_0 values. Consistent with previous studies (3–8) the data of Table IV show that:

$$k_0 = k_0' - k_cC \qquad \text{(Eq. 16)}$$

where $k_0' = 5.10 \times 10^{-4}$ sec.$^{-1}$ is the first-order growth rate constant in the absence of chloramphenicol and $k_c = 2.68 \times 10^{-4}$ ml./mcg./sec. is the inhibitory constant where C is in mcg./ml.

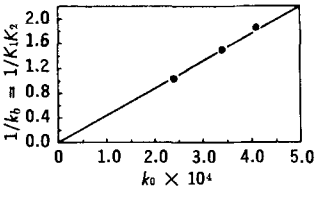

Fig. 9—The product ($1/K_1K_2$) of sulfonamide partition constant ($1/K_1$) and the sulfonamide-receptor site dissociation constant ($1/K_2$) as a function of the growth rate constant k_0 in the presence of chloramphenicol at 37.5°.

The action of chloramphenicol on microbial growth has been assigned to the inhibition of the function of messenger RNA in protein synthesis by blocking its attachment to ribosomes through competition for ribosomal binding sites (33). The differences in the kinetics in regard to lag period, reversibility, and concentration dependence are indicative that chloramphenicol and sulfonamide mechanisms of action differ.

Since $1/K_2$ is directly proportional to R and if the numbers of receptor sites, R, are a function of the microbial growth rate, it follows that $1/K_1K_2$ should be a linear function of k_0, the growth rate constant in the presence of chloramphenicol and in the absence of sulfisoxazole. Since the data (Table IV) are at constant temperature, the partition constant, K_1, should be invariant.

The plot of the data in Fig. 9 confirms this reasoning since it demonstrates that a linear relation with an intercept of zero exists and:

$$1/K_1K_2 = mk_0 \qquad \text{(Eq. 17)}$$

where $m = 0.440$.

For predictive purposes, we can describe the number of microorganisms at any time, t, when chloramphenicol and sulfonamide are administered simultaneously as:

$$N = N_1 e^{k_{app.}(t - t_1)} \qquad \text{(Eq. 18)}$$

where N_1 is the number of *E. coli* in balanced growth at time t_1, where within the first 5.5 generations $k_{app.}$ is defined by k_0 in Eq. 16. When the new sulfonamide-affected steady state is achieved, $k_{app.}$ is defined from consideration of Eqs. 12, 13, and 16 as:

$$k_{app.} = \frac{[k_0' - k_cC][1 + K_1K_2(S - 1)]}{1 + K_1K_2 S} \qquad \text{(Eq. 19)}$$

where k_0, k_c, K_1, and K_2 have been shown in this paper and others (7, 8) to conform to the Arrhenius–Van't Hoff relation over a considerable temperature range:

$$k = P_e - \Delta H/RT \qquad \text{(Eq. 20)}$$

For a given temperature where m in Eq. 17 is known, Eq. 19 can be written as:

$$k_{app.} = k_0 - 1/(m + S/k_0) \qquad \text{(Eq. 21)}$$

REFERENCES

(1) Garrett, E. R., Ågren, A. J., and Lambert, H. J., *Intern. J. Clin. Pharm.*, **1**, 1(1967).
(2) Garrett, E. R., *Antibiot. Chemotherapy*, **8**, 8(1958).
(3) Garrett, E. R., and Brown, M. R. W., *J. Pharm. Pharmacol.*, **15**, 185T(1963).
(4) Brown, M. R. W., and Garrett, E. R., *J. Pharm. Sci.*, **53**, 179(1964).
(5) Garrett, E. R., and Miller, G. H., *ibid.*, **54**, 427(1965).
(6) Garrett, E. R., Wright, O. K., Miller, G. H., and Smith, K. L., *J. Med. Chem.*, **9**, 203(1966).
(7) Garrett, E. R., Miller, G. H., and Brown, M. R. W., *J. Pharm. Sci.*, **55**, 593(1966).
(8) Garrett, E. R., *Arzneimittel-Forsch.*, **16**, 1364(1966).
(9) Kohn, H. I., and Harris, J. S., *J. Pharmacol. Exptl. Therap.*, **73**, 343(1941).
(10) Harris, J. S., and Kohn, H. I., *ibid.*, **73**, 383(1941).
(11) Cowles, P. B., *Yale J. Biol. Med.*, **14**, 599(1942).
(12) Brueckner, A. H., *ibid.*, **15**, 813(1943).
(13) Campbell, A., *Bacteriol. Rev.*, **21**, 263(1957).
(14) Harvey, R. J., Marr, A. G., and Painter, P. R., *J. Bacteriol.*, **93**, 605(1967).
(15) Anton, A. H., *J. Pharmacol. Exptl. Therap.*, **129**, 282 (1960).
(16) Powell, E. O., and Errington, F. P., *J. Roy. Microscop. Soc.*, **82**, 39(1965).
(17) Northey, E. H., "The Sulfonamides and Allied Compounds," Reinhold Publishing Co., New York, N. Y., 1948.
(18) Woods, D. D., *Brit. J. Exptl. Pathol.*, **21**, 74(1940).
(19) Woods, D. D., and Fildes, P., *Chem. Ind. (London)*, **59**, 133(1940).
(20) Fildes, P., *Lancet*, **I**, 955(1940).
(21) Woods, D. D., *Ann. N. Y. Acad. Sci.*, **52**, 1197(1950).
(22) Brown, G. M., *J. Biol. Chem.*, **237**, 536(1962).
(23) Seydel, J. K., Krüger-Thiemer, E., and Wempe, E., *Tuberk.-Forschungsinst. Borstel, Jahresber.*, **5**, 651(1961).
(24) Seydel, J. K., *Antibiot. Chemotherapia*, **12**, 135(1964); Seydel, J. K., and Wempe, E., *Arzneimittel-Forsch.*, **14**, 705 (1964).
(25) Muir, R. D., Shaleffer, V. J., and Jones, L. R., *J. Bacteriol.*, **44**, 95(1951).
(26) Nimms-Smith, R. H., Lascelles, J., and Woods, D. D., *Brit. J. Exptl. Pathol.*, **29**, 264(1948).
(27) Lascelles, J., and Woods, D. D., *ibid.*, **33**, 288(1952).
(28) White, P. J., and Woods, D. D., *J. Gen. Microbiol.*, **40**, 243(1965).
(29) Rose, H. M., and Fox, C. L., *Science*, **95**, 412(1942).
(30) MacLeod, C. M., *J. Exptl. Med.*, **72**, 217(1940).
(31) Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F., *Science*, **97**, 265(1943).
(32) White, P. J., and Woods, D. D., *J. Gen. Microbiol.*, **40**, 243(1965).
(33) Weisberger, A. S., and Wolfe, S., *Federation Proc.*, **23**, 976(1964).