sium into the red blood cells of human beings is relatively low compared with that of other tissue cells(3). Provided a total body cell K/red cell K ratio of 15 the calculated minimal 24 hour total cellular potassium uptake would be about 14 g (358 mEq) K. Actually this patient in a well controlled period prior to the experiment received 23 g (588 mEq) K during 2 days; within this period the potassium concentration of plasma increased from 2.28 to 3.45 mEq/L and of the red blood corpuscles from 94.4-99.8 mEq/L. The potassium concentration of the muscular tissue was extremely low at the time of the experiment: 50.9 mEq/kg; Na:107 mEq/kg.

Summary. The potassium exchange of normal human erythrocytes *in vitro* increased with increasing plasma potassium concentration and approached a constant level at concentrations above 9 mEq/L.

In an experiment with low potassium erythrocytes from a case of persistent hypopotassemia in which the plasma potassium concentration was raised the potassium uptake (K-influx) was found to be increased by 40% of the corresponding values for normal erythrocytes. This increase in the K-influx corresponded quantitatively to a demonstrated net transport of potassium into the low potassium red blood cells. The simultaneous emigration of K-ions, however, was not reduced, and therefore the net transport of potassium could not be considered a result of a reduced potassium emigration from the cells.

The low potassium concentrations of the erythrocytes in this particular case seemed to reflect the potassium depletion of the body cells.

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In Vitro Sensitivity of Bacteria to Sulfonamide Combinations as Compared to Single Sulfonamides.* (17946)

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The therapeutic advantages of a mixture of several sulfonamides over a single effective sulfonamide are said to be due to the following: (1) A given volume of fluid (*e.g.* urine) is capable of retaining several sulfonamides in solution up to their individual maximum solubilities, so that larger doses of a mixture than the maximum for any one sulfonamide may be given without producing crystalluria. (2) On a weight for weight basis, such mixtures in a large series of cases have been given with a markedly reduced incidence of renal toxicity(1-9) and of sensitization reactions such as drug rash and fever(7). It is claimed further that mixtures are as effective antibacterial agents *in vitro* as an equivalent amount of a single sulfonamide and may in some instances exert a potentiat-

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ing effect(8,9). While the reported clinical results with mixtures appear satisfactory, the clinical and laboratory data demonstrating that mixtures on a weight for weight basis possess equal or greater antibacterial potency than single effective sulfonamides is meagre or altogether lacking. Since the degree of sensitivity *in vitro* of a given strain of bacteria to single sulfonamides is a useful index of the clinical response(10), similar data may be expected to hold true for sulfonamide combinations as well. This communication presents data on the comparative bactericidal effects of a given dose of a mixture and of its individual components.

Material and methods. The *in vitro* sensitivity of bacteria to sulfonamides in culture media varies with the inhibiting properties of certain constituents in the media. To obtain the absolute range of antibacterial concentrations of a sulfonamide, it is necessary to prepare culture media completely free of inhibitors. White *et al.*(11), Kohn and Harris(12,13) and others have made use of such media. They showed that while inhibitors lower the absolute antibacterial power of sulfonamides, they do not affect their relative potency on the same strain. Since the preparation of inhibitor-free media has not been standardized or made practicable for clinical purposes, we developed a simple technic for testing sensitivity(10) which utilizes peptone broth (Difco). Although the large quantity of inhibitors in peptone broth necessitates the use of bactericidal concentrations of sulfonamides up to 250 mg % to indicate clinical effectiveness(10), the method provides a valid index of the most appropriate sulfonamide for clinical use in a given case. In the table below, the effective bactericidal concentrations should be interpreted with the above considerations in mind.

I. *Micro-organisms.* Seventy bacterial strains† were studied *in vitro.* Twenty-six were gram-positive cocci, as follows: hemolytic *Staphylococcus aureus* 6; non-hemolytic *Staphylococcus aureus* 4; hemolytic streptococci 8; non-hemolytic streptococci 8. Forty-four were gram-negative strains, as follows: *Escherichia coli* 6, *Aerobacter aerogenes* 6, *B. proteus vulgaris* 6, *Pseudomonas aeruginosa* 6, *Klebsiella Friedlander* Type A 2, and Type B 2; and 16 strains of the various Shigellae (*Shigella shigae,* 4; *Shigella dispar,* 2; *Shigella sonnei,* 2; *Shigella alkalescens,* 2; *Shigella ambigua,* 1; *Shigella paradysenteriae,* types V,W,Y,Z, and Newcastle) one strain each.

II. *Sulfonamide drugs.* The following sulfonamides were used in the *in vitro* tests: sulfadiazine (SD), sulfamerazine (SM), sulfamethazine (SMT), sulfathiazole (ST). They were used singly or combined in equal amounts by weight as follows: sulfadiazine and sulfamerazine; sulfadiazine and sulfathiazole; sulfamethazine and sulfathiazole; sulfadiazine, sulfamerazine and sulfamethazine; sulfadiazine, sulfamerazine and sulfathiazole.

III. *Technic.* 1000 mg % stock solutions of each sulfonamide in broth were prepared and brought to a pH of 8.2. Stock solutions of the sulfonamide combinations were prepared by mixing equal volumes of stock solutions of the individual sulfonamides. Serial dilutions of each of these stock solutions were prepared in concentrations of 750, 500, 333, 250, 100, 50, 25, and 10 mg % respectively. One cc of each of the 8 dilutions was placed in sterile Wassermann tubes. To each tube was added 0.1 cc of a bacterial suspension prepared by diluting a 24 hour culture so that 0.1 cc contained 500-5000 bacteria. A control tube containing broth without sulfonamide was also inoculated with 0.1 cc of the same bacterial suspension. All

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† All cultures except the Shigellae were strains freshly isolated from patients. The Shigellae were old laboratory strains received from the American Type Culture Collection. A large number of the latter were tested because the sulfonamides are especially useful in the treatment of bacillary dysentery.

TABLE I.

Patterns of Response of 70 Bacterial Strains to Varying Concentrations (in mg %) of Single Sulfonamides and Sulfonamide Mixtures.

Patterns of response* Type	Strains	Drugs									Comment
		SD	SM	SMT	ST	SD+SM	SD+ST	SMT+ST	SD+SM+SMT	SD+SM+ST	
A	10	100	100	100	50	250	100	100	333	250	No additive action* in mixtures
B	11	250	250	250	500	250	333	333	250	333	Additive action in mixtures
C	14	>1000	>1000	>1000	250	>1000	500	>1000	>1000	750	Action of mixtures due exclusively to ST
D	8	>1000	>1000	>1000	100	>1000	250	250	>1000	500	Same
E	17	>1000	>1000	250	250	>1000	500	500	>1000	750	No additive action in mixtures
F	10	250	250	100	100	250	50	50	250	100	Potentiating action† in mixtures (SD+ST, SMT+ST)

* Additive action indicates that the bactericidal action of the mixture was equal to the sum of the action of its components.
† Potentiating action indicates that the bactericidal action of the mixture was greater than the sum of the action of its components.

tubes were incubated at 37°C for 24 hours (or longer in the case of streptococci). The lowest concentration of each sulfonamide or sulfonamide mixture, which completely inhibited macroscopically visible growth, was taken to be the bactericidal titer,‡ which is the only titer that may be considered a reliable guide in assessing the probable clinical effectiveness of a sulfonamide(10). All tests were run in duplicate.

Results. The results were classifiable into 6 patterns of response to varying concentrations of the individual sulfonamide (Table I). The bactericidal titers for the strains within a given type varied slightly, but not significantly, from the pattern shown in the Table. In the first type of response (A, Table I), the bactericidal titer of SD alone and of SM alone was 100 mg %. But the bactericidal titer of a mixture of equal parts of these two drugs was 250 mg %, indicating that the bactericidal action of the mixture was less than that of either drug alone. The same was true of a mixture of equal parts of SD, SM, and SMT, which required 333 mg % to produce a bactericidal effect. In the case of a mixture of SD and ST, the bactericidal titer was 100 mg % whereas for ST alone it was 50 mg %. There was no additive effect from the SD present. The same was true of a mixture of SMT and ST. Since a mixture of SD, SM, and ST required a concentration of 250 mg %, it is evident that not only was there no additive effect, but some suppression of sensitivity to ST may have been produced.

These data indicate for these 10 strains that at optimum concentration of a given effective sulfonamide, adding another or several others not only provides no advantage, but the reverse may occur, *i.e.*, the adequacy of a given concentration of a sulfonamide acting alone may be impaired by adding another. The ten strains in this group consisted of 3 gram-positive cocci (2 staphylococci, 1 beta hemolytic streptococcus) and 7 gram-negative bacilli.

‡ Subcultures did not reveal any growth from any clear tube, the sulfonamide concentration of which was taken to be the bactericidal titer.

In the Type B response, the bactericidal titer of some double or triple mixtures indicated an additive effect, since the concentration of each of the sulfonamides in the mixture, when acting alone, was not bactericidal. The effective titer was in a range which we would estimate to be clinically effective. All the strains in this group were gram-positive cocci: 4 staphylococci, 3 hemolytic and 4 non-hemolytic streptococci.

The character of the C & D types of response was a clinically effective sensitivity to one sulfonamide only, ST, and resistance to 3 others. The bactericidal effect of the sulfonamide mixtures was due exclusively to this one effective sulfonamide in the mixture in the same concentration required when it was acting alone. The 22 strains in these 2 types of response were 2 strains of staphylococci, 2 non-hemolytic streptococcus and 18 strains of gram negative bacilli, which included all species of gram-negative bacilli tested.

In Type E there was no evidence of an additive or potentiating influence in the various mixtures. All 17 strains in this class were gram-negative bacilli and were in a range indicative of clinical effectiveness to only 2 of the four sulfonamides tested.

In Type F the bacteria were weakly sensitive to some sulfonamides and very sensitive to others. There was a definite additive effect in some mixtures, (SD and SM), and a potentiating effect in others (SD and ST, SMT and ST). Most of the organisms were gram positive cocci, (2 strains of staphylococci, 4 of hemolytic and 2 of non-hemolytic streptococci, and one strain each of *E. coli* and *A. aerogenes*).

Discussion. An additive or potentiating effect of mixtures occurred in 21 of the 70 strains tested. Nineteen of the 21 were gram-positive cocci. A potentiating effect was also observed in one strain of *E. coli* and in one of *A. aerogenes*. No additive or potentiating effect occurred in 42 of the 44 strains of gram-negative bacilli tested. As already pointed out(10), variations in *in vitro* sensitivity to several sulfonamides are not large in the case of most cocci, but may be very considerable in the case of gram-negative bacilli. If the close correlation between *in vitro* sensitivity to single sulfonamides and therapeutic effect(10,14) also holds for mixtures, our data suggest that the favorable effect of mixtures would be expected in the case of the majority of coccal infections but not for most gram-negative bacillary infections. A favorable clinical effect of mixtures particularly in coccal infections has been reported by Lehr and others(3,4,6,9). But the effects of mixtures as compared to single sulfonamides awaits more searching clinical evaluation than is evident from the literature to date. Since (a), 27% of the gram-positive cocci and 95% of the gram-negative bacilli which we tested were less sensitive to mixtures than to single component sulfonamides, in what we judge to be a clinically effective range of concentration, and (b), individual strains of a given bacterial species may be less, equally, or more susceptible to mixtures than to single components of the mixture on a weight for weight basis, [see also Seneca *et al.* (11)] the choice between a mixture and a single sulfonamide requires determination in the individual case. On the evidence so far available, the superiority of a mixture cannot be assumed and is in fact unlikely in the case of most gram-negative bacillary infections.

Conclusion. The use of sulfonamide mixtures in preference to a single effective sulfonamide is justified only when *in vitro* sensitivity tests demonstrate for the components of the mixture either an additive or a potentiating effect. Such tests suggest that this is likely to be true in the majority of coccal infections, but not for gram-negative bacillary infections.

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