made a thorough investigation of the course of infection of trypanosomiasis in animals and chick embryos with the same strains of organisms used in this study.

Summary. White mice and chick embryos were infected with *T. brucei, T. equiperidum* and *T. hippicum.* After a lapse of 24-36 hours streptomycin was administered subcutaneously to the mice and was injected into the yolk sac of the chick embryos. Each mouse received a total of 16,000 units and each chick embryo, a total of 40,000 units of streptomycin. The course of the disease was followed by microscopic examination of blood specimens taken at regular intervals. As far as could be determined, this antibiotic agent did not alter the course of the infections or prolong the life of the treated mice or embryos.

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Low Toxicity of Sulfonamide Mixtures. II. Combinations of Sulfathiazole, Sulfadiazine and Sulfamerazine.*

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A new and simple approach to the prevention of renal complications caused by sulfonamides was presented in a previous communication.[1] It consists in the use of *mixtures* of sulfonamides instead of single compounds. The idea emerged from the observation that a saturated aqueous or urinary solution of a sulfonamide could still be fully saturated with a second and third sulfonamide of different molecular structure, each compound behaving as though it were present alone and exerting no influence on the solubilities of the others. Consequently, in solutions containing several sulfonamides, the maximum obtainable concentration appeared expressed by the sum of the solubilities of all the drugs present. This finding applied to the free compounds, as well as to their acetylated homologues.

It was reasoned on the basis of this observation that the danger of intrarenal formation of sulfonamide crystals could be considerably reduced by employing combinations of *partial* dosages of 2 or more therapeutically equivalent sulfonamides instead of single compounds. The validity of this contention was demonstrated in experimental and clinical studies with a mixture of sulfathiazole and sulfadiazine.[2,3]

The present paper deals with the toxicity of a sulfadiazine-sulfamerazine and a sulfathiazole-sulfadiazine-sulfamerazine combination. It contains also some additional data on the sulfathiazole-sulfadiazine mixture, in particular a comparative quantitative estimation of intrarenal sulfonamide deposits from sulfathiazole, sulfadiazine, and their combination.

Materials and Methods. Four hundred albino rats from our own colony, 8-12 weeks old and weighing between 160-210 g were employed in all experiments with the exception of the study on chronic toxicity in which weanling rats were used. The animals were kept on a standard diet (Rockland Farms Rat Diet) and had free access to water.

For determination of the acute toxicity, observation was continued for 5 days following a single intraperitoneal injection of the sulfonamides because of the well known de-

* This investigation has beeen aided by grants from the Josiah Macy, Jr., Foundation and the Schering Corporation, Bloomfield, N.J.

1 Lehr, D., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 11.

2 Lehr, D., *J. Urol.*, 1946, **55**, 548.

3 Lehr, D., Slobody, L. B., and Greenberg, W. B., *J. Pediat.*, 1946, **29**, 275.

TABLE I.
Comparative Acute Toxicity in Male Albino Rats of Sodium Salts of Sulfadiazine (NaSD), Sulfathiazole (NaST), Sulfamerazine (NaSMD), and Mixtures of 2 and 3 of These Compounds.

Drug, g/kg body wt: Single intraperitoneal inj. of	Sulfonamide total amt	No. of animals	No. of dead	% dead	Died within days
NaSD, 1.5	1.5	90	76	85	2-4
NaST, 1.1	1.1	25	16	65	1-4
NaSMD, 1.5	1.5	29	15	52	1-3
NaSD, 0.75 NaST, 0.55	1.3	60	7	12	2-4
NaSD, 0.75 NaSMD, 0.75	1.5	30	11	37	1-2
NaSD, 0.54 NaST, 0.42 NaSMD, 0.54	1.5	30	1	3	1-2

layed death from sulfadiazine, and to some extent also from sulfamerazine intoxication. As a rule 5% aqueous solutions of the sodium salts of sulfathiazole, sulfadiazine, and sulfamerazine were employed. Mixtures of two drugs were prepared conveniently by mixing equal parts of the 5% solutions so that the final concentration was 2.5% each of sulfadiazine and sulfathiazole, or sulfadiazine and sulfamerazine. If unequal amounts of 2 or 3 drugs were used, as in the acute toxicity study with the sulfathiazole-sulfadiazine mixture, the initial concentrations of the individual drugs were so adjusted, that mixing of equal fluid volumes resulted in the desired final concentrations of the 2 or 3 components. Thus identical amounts of fluid were injected in all groups. In each test the toxicity of combined sulfonamides was evaluated *simultaneously* with the toxicity of equal or comparable dosages of the separate components of the mixture.

Ten rats represented the minimum experimental unit. Subgroups of 5 animals each were placed into separate metabolism cages. The sulfonamide concentration in the blood was determined from the tail vein of each animal, at least at one occasion, in order to eliminate rats injected inadvertently into the gut instead of intraperitoneally. In some experiments the sulfonamide level in blood and urine was followed for 48 hours.

In other studies groups of animals were killed at predetermined intervals of a 48-hour period. The blood was used for estimation of the sulfonamide and nonprotein nitrogen level. The entire urinary tract was carefully inspected for the presence of crystalline deposits. The kidneys were weighed and cross-sections examined under the lens for concrements in the pelvis and for intratubular drug precipitation. The sulfonamide concentration was then determined in the kidneys according to a procedure previously outlined in detail.[4]

The subacute toxicity was studied by repeated intraperitoneal injections of sublethal dosages of the sulfonamides and their mixtures. The level of nonprotein nitrogen and sulfonamide in the blood was determined repeatedly and the total daily drug excretion in the urine was followed throughout the entire experimental period.

The chronic toxicity was determined by incorporating the sulfonamides in various concentrations and combinations into the powdered form of the standard diet. The experiments were conducted with weanling rats weighing 60-80 g and planned for a period of 6 weeks. The food consumption was checked daily. The body weight of each animal was recorded twice weekly. Blood concentration and total urinary elimination of sulfonamide were determined at the same time intervals. The nonprotein nitrogen levels in the blood were estimated upon termination

[4] Lehr, D., and Antopol, W., *Urol. and Cutan. Rev.*, 1941, **45**, 545.

TABLE II.
Findings in Blood and Kidneys 24 Hours After a Single Intraperitoneal Injection of Sulfonamides in Albino Rats.

Group No.	Drug, g/kg	Blood			Kidney (moist tissue)				
		N.P.N., mg %	Total sulfa, mg %	% acetylation	Wt (both kidneys)	Total sulfa		% acetylation	Appearance of renal cross-section
						mg %	mg		
1	NaSD, 1.5	197	140	19	2287	454	*10.3*	23	Considerable amt of drug ppt. in entire medulla. Marked edema.
2	NaST, 1.1	154	62	27	2137	160	*3.4*	40	Same in the papilla and pelvis. Marked edema.
3	NaSD, 0.75 / NaST, 0.55	104	30	7	1763	34	*0.6*	6	No ppt. visible in 2 animals. Trace of ppt. in 1 animal. Slight edema.
4	NaSD, 0.75	143	51	22	2259	89	2.0	38	Ppt. obstructs the papilla. Marked edema.
5	NaST, 0.55	53	1	23	1436	0	0	0	No ppt. visible. No edema.
6	Controls	**48**			1472				

All figures represent the mean of the values from 3 female animals.

of each experiment and correlated with the pathologic-anatomical findings in the kidneys.

In all experiments, the surviving rats were killed by exsanguination in ether narcosis. Postmortem examinations were performed on these animals and as far as feasible also on rats succumbing during an experiment. Special attention was given to changes in the urinary tract. The most important organs of representative animals were fixed in formaldehyde for histological study.

Determination of free and conjugated sulfonamide was carried out according to the method of Bratton and Marshall[5] using a Klett Summerson photo colorimeter. All figures of drug concentration were expressed in terms of the free sulfonamides and not of their sodium salts. Obviously no differentiation could be made between sulfathiazole, sulfadiazine and sulfamerazine in determinations of mixtures in body fluids. However, these compounds have very similar molecular weights and, therefore, give almost identical colorimetric readings.

Results. 1. *Acute Toxicity.* A condensation of the most important results from the acute toxicity studies is presented in Tables I and II and in Fig. 1.

Sulfadiazine-Sulfathiazole Mixture. It is apparent from Table I that the extensive increase in the number of animals in the sulfathiazole-sulfadiazine experiment did not result in any significant change in the mortality figures as compared to those originally reported.[1]

It was inferred from these results that the danger of renal blockage can be significantly reduced by the joint administration of sulfadiazine and sulfathiazole in *partial* dosages. Added proof for the validity of this viewpoint was derived from a series of experiments conducted to determine the accurate amounts of sulfonamide present in the kidneys 4 hr., 8 hr., 24 hr., and 48 hr. after a single intraperitoneal injection of the sulfadiazine-sulfathiazole mixture as compared to either compound when injected separately.

It was found that intrarenal precipitation

[5] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

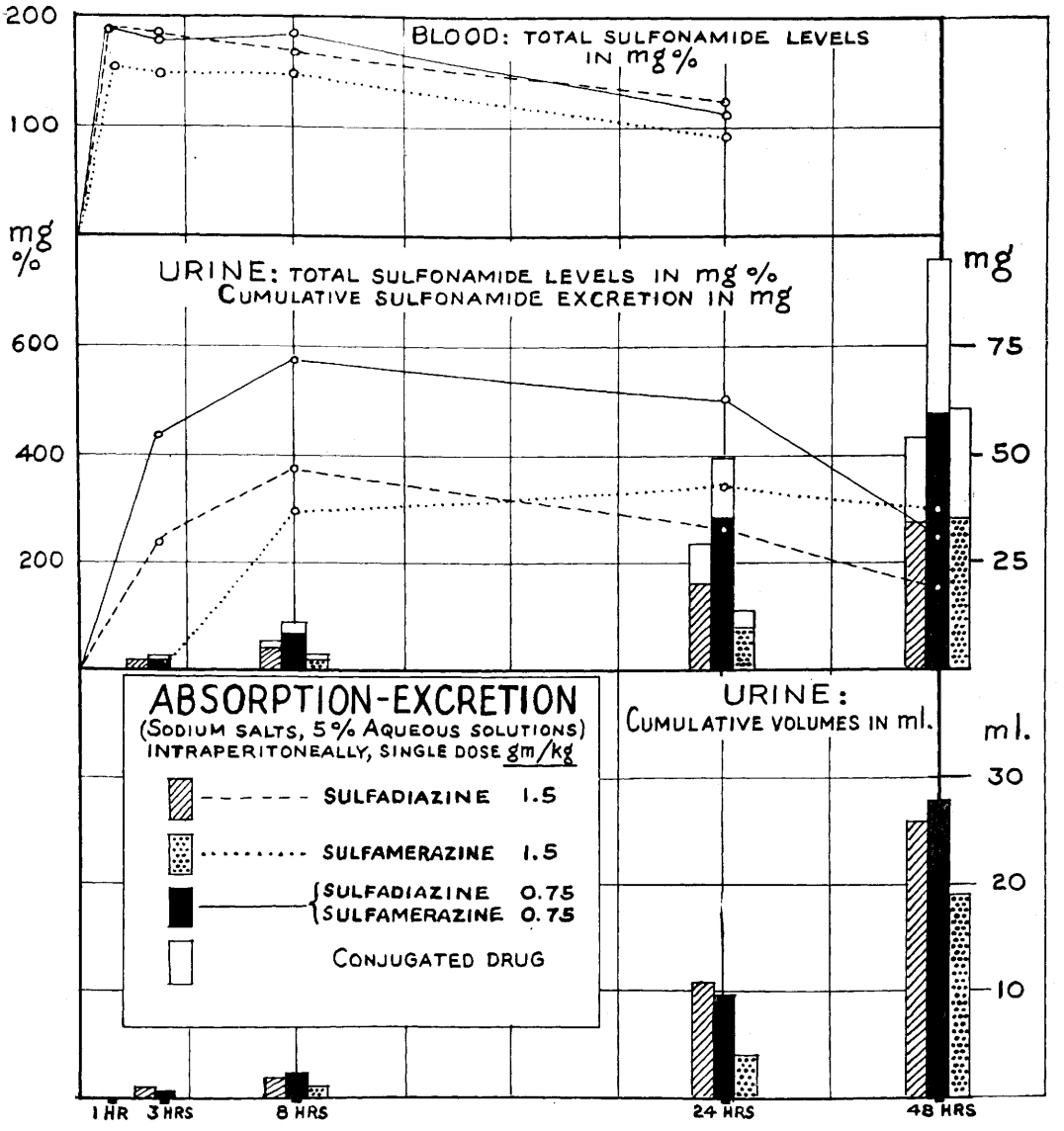

FIG. 1.

Graphic representation of the comparative absorption and excretion of sulfadiazine, sulfathiazole and a mixture of these two compounds in albino rats.

of sulfonamide crystals occurred initially in all groups, even in those receiving only *half* the dosages of either sulfathiazole or sulfadiazine alone. But there were highly significant differences in the quantity and duration of intratubular crystalline deposition.

Table II summarizes one representative experiment. The table demonstrates that after 24 hours the rats injected with the sulfadiazine-sulfathiazole mixture (Group 3) were practically free of renal drug deposits. Actually the kidneys of these animals contained less than one-tenth the amount of sulfonamide expected on the basis of the values from the full dosages of the separate compounds (italic figures), and even this small amount is fully accounted for by the general tissue concentration, as apparent from the sulfonamide blood level.

It was interesting to note that half the dose of sulfadiazine resulted in more precipitation when administered separately than

TABLE III.
Urinary Elimination of Single and Mixed Sulfonamides After One Intraperitoneal Injection in Albino Rats.
(Each value represents the mean from at least 10 animals.)

Drug	Dose, g/kg		% of administered dose excreted with urine in:		
	Partial	Total	8 hr	24 hr	48 hr
NaSD		1.5	1.9	9.6	16.0
NaSMD		1.5	1.2	5.4	24.0
NaSD	.75	1.5	4.7	21.0	40.0
NaSMD	.75				
NaSD	.54				
NaSMD	.54	1.5	9.0	39.0	50.0
NaST	.42				

TABLE IV.
Comparative Subacute Toxicity in Male Albino Rats of Sodium Salts of Sulfadiazine (NaSD), Sulfathiazole (NaST), Sulfamerazine (NaSMD), and Mixtures of 2 and 3 of These Compounds.
(The mortality figures are shown as they appeared when 100% mortality was reached in the sulfathiazole group, that is, after 4 injections.)

Drug, g/kg body wt				
Daily intraperitoneal inj. of	Sulfonamide total amt. inj.	No. of animals	% death	Died within days
NaSD, .9	3.6	15	87	2-5
NaST, .9	3.6	15	100	1-4
NaSMD, .9	3.6	20	55	3-5
NaSD, .45 + NaST, .45	3.6	10	30	3-4
NaSD, .45 + NaSMD, .45	3.6	10	0	
NaSD, .3 + NaST, .3 + NaSMD, .3	3.6	10	0	

when given in combination with sulfathiazole. This paradoxical phenomenon can be explained by the greater diuresis in the presence of higher sulfonamide concentrations in the tubular urine. It is obvious that sulfonamide mixtures retain the same osmotic value as equal concentrations of single compounds. Thus animals receiving the partial dose of only *one* component of the mixture are exposed to no less danger of oversaturation in the tubular urine, while at the same time they are deprived of the greater diuretic effect of higher sulfonamide concentrations as present in the urine of mixture animals.

Mixtures of Sulfadiazine-Sulfamerazine and Sulfadiazine-Sulfathiazole-Sulfamerazine. The low toxicity of these 2 combinations is illustrated in Table I. It can be seen that a mixture of 3 sulfonamides is significantly less toxic than a mixture of 2 compounds.

The absorption and excretion of sulfonamide combinations is exemplified in Fig. 1. It is evident that a mixture of half dosages of sulfadiazine and sulfamerazine is more completely absorbed and excreted than either compound administered separately in the same total dosage. Similar observations were made with the mixture of sulfadiazine-sulfathiazole and the combination of all 3 sulfonamides. The latter gave the most complete urinary recovery figures.

In this connection, it should be kept in mind that excessive dosages of sulfonamides, which result in renal obstruction, are neces-

TABLE V. Body Weight, Nonprotein Nitrogen and Sulfonamide Levels in Blood, and Pathologic-Anatomical Findings in Kidneys of Albino Rats Surviving After 6 Weeks on a Diet Containing Either Sulfadiazine, Sulfamerazine, or a Mixture of the 2 Drugs.

% drug in the diet	No.	Sex	Body wt	Blood N.P.N. mg %	Blood Sulfonamide, mg %	Findings in the kidneys	Remarks
3% SD	1	F	88	103	33	Parenchymatous damage in all survivors with calcifying nephrosis in 3 instances	1 died within 5 wk
	2	F	76	101	24		
	3	F	93	145	37		
	4	F	76	121	37		
	5	F	80	126	31		
	6	M	115				
	7	M	110	149	34		
	8	M	108	93	30		
	9	M	113	107	27		
		Avg	95	118	31		
3% SMD	2	F	63	89	56	Many concrements throughout entire urinary tract in one survivor; gravel in the pelves and intrarenal precipitate in the other four	4 died within 4 wk
	5	F	76	103	57		
	8	M	84	110	53		
	9	M	60	84	69		
	10	M	80	64	54		
		Avg	73	90	58		
1½% SD 1½% SMD	1	F	78	70	64	Traces of gravel in 4 of 9 survivors; otherwise normal kidneys	1 died within 2 wk
	2	F	75	45	55		
	3	F	77	84	45		
	4	F	68	67	64		
	5	F	78	45	49		
	7	M	84	75	62		
	8	M	74	73	63		
	9	M	80	72	63		
	10	M	88	61	64		
		Avg	78	66	59		
Controls	1	F	148	53			
	2	F	144	47			
	3	F	166	44			
	4	F	150	46			
	5	F	160	34			
	6	M	248	45			
	7	M	202	58			
	8	M	192	54			
	9	M	200	46			
	10	M	215	50			
		Avg	183	48			

sarily much less completely eliminated with the urine than small amounts. Hence high recovery figures in the urine, as observed with sulfonamide combinations, can mean only diminished interference with renal elimination. In line with this interpretation, Table III indicates that the percentage of urinary recovery is directly proportional to the number of sulfonamides in the combination, since any addition to this number results necessarily in smaller partial dosages of each component.

2. *Subacute Toxicity.* The results of the studies in male albino rats are summarized in Table IV. All 3 sulfonamide combinations are again conspicuous because of their low toxicity.

A comparison with Table I reveals that the results of the subacute experiments were in good agreement with those of the acute study. This applied also to levels of nonprotein nitrogen and sulfonamide in the blood as well as to urinary drug excretion and autopsy findings. Identical acute and subacute toxicity studies with *female* rats were confirmatory, although female animals proved in general distinctly more sensitive to the sulfonamides.

3. *Chronic Toxicity.* Studies with a sulfathiazole-sulfadiazine food mixture had to be abandoned because weanling rats submitted to almost complete voluntary starvation at sulfathiazole and at sulfathiazole-sulfadiazine concentrations of 5% and even 3% in the diet.

Sulfamerazine, although still less well tolerated than sulfadiazine, permitted an intake at the 3% level in the food sufficient both for the survival of the rats and for the creation of serious renal lesions. It should be stressed that at the same concentration the sulfadiazine food was consumed in larger amounts; accordingly, the voluntary intake of the sulfadiazine-sulfamerazine combination was in between the one of either compound given separately. The end result of a 6-week feeding study is shown in Table V.

Measured in terms of survival and inhibition of growth, sulfamerazine would appear to be more toxic than sulfadiazine and the combination. In comparing sulfadiazine and the sulfadiazine-sulfamerazine mixture one might be misled by the greater retardation of growth from the latter into considering the mixture more toxic than sulfadiazine. However, if one compares the nonprotein nitrogen and sulfonamide levels in the blood as well as the findings in the kidneys, it becomes immediately apparent that sulfadiazine like sulfamerazine is by far more toxic than the combination of the 2 drugs.

The less significant growth inhibition in the sulfadiazine group was obviously due to the higher food consumption. Despite the simultaneously greater drug intake, the blood levels from sulfadiazine were throughout the experiment lower than from sulfamerazine and the combination. Since there was little difference in the total urinary excretion of sulfadiazine and sulfamerazine, it is obvious that sulfadiazine was slower and less completely absorbed from the gastro-intestinal tract and faster excreted by the kidneys than sulfamerazine. Renal damage was very marked in the sulfadiazine as well as in the sulfamerazine group, whereas it was insignificant in the animals fed the combination of the 2 drugs.

The fact that sulfamerazine may produce more concrement formation than a higher dose of sulfadiazine should serve as a warning that renal complications may occur from sulfamerazine at least as readily as from sulfadiazine. Although this finding is in contrast to previous experimental observations,[6,7] it is strongly supported by growing clinical experience.[8,9]

Discussion. The present study extended the experimental evidence for the strikingly low toxicity of sulfonamide combinations from the sulfathiazole-sulfadiazine mixture

[6] Welch, A. D., Mattis, P. A., Latven, A. R., Benson, W. M., and Shields, E. H., *J. Pharm. and Exp. Therap.*, 1943, **77**, 357.

[7] Schmidt, L. H., Hughes, H. B., and Badger, E. A., *J. Pharm. and Exp. Therap.*, 1944, **81**, 17.

[8] Flippin, H. F., and Reinhold, J. G., *Ann. Int. Med.*, 1946, **25**, 433.

[9] Hageman, P. O., Harford, C. G., Sobin, S. S., and Ahrens, R. E., *J. A. M. A.*, 1943, **123**, 325.

to include combinations of sulfadiazine-sulfamerazine, and sulfathiazole-sulfadiazine-sulfamerazine. Confirmatory experimental results were reported recently by other investigators.[10,11] In this connection it is of importance to stress that the toxicity of sparingly soluble sulfonamides is dependent upon 2 factors. The one, representing the true tissue toxicity, consists in the direct chemical action of the sulfonamides on the living cell; the other, as a function of solubility and renal clearance of these compounds, lies in the hazard of mechanical blockage in the urinary tract.

Lately the factors influencing the prevention and control of renal blockage from sulfonamides were summarized in a lucid review by Scudi.[12] The significance of renal obstruction in the over-all toxicity of heterocyclic derivatives of sulfanilamide was demonstrated in the animal experiment.[4,13] It was also shown that measures which diminish the intrarenal formation of sulfonamide crystals[14] or remove deposits from the tubules[15] will result in a most significant drop in the mortality. The low toxicity of sulfonamide combinations can, therefore, be interpreted in the light of experimental proof for a strongly diminished tendency to intrarenal precipitation, as due mainly to a decrease in the renal factor of toxicity. This viewpoint is strengthened by the lack of evidence for any other mechanism of detoxification, since one would not expect pronounced changes in the true cellular toxicity of closely related compounds if they retain their full antibacterial activity in combinations.

In line with this interpretation was the further significant lowering of the toxicity for a mixture of *3* sulfonamides. It was obviously due to the fact that the triple mixture contained necessarily *smaller* partial dosages of the individual compounds, resulting in a further diminution of the possibility of oversaturation and hence precipitation in the tubular urine.

Since at the bedside crystalluria was strongly decreased, even with mixtures of *2* sulfonamides and in the absence of alkalization,[2,3,8] it seems justified to assume that one might safely dispense with the burden and disadvantages[8,16] of adjuvant alkali therapy when employing combinations of *3* sulfonamides.

The unexpectedly high blood and urine levels obtained with mixtures, as well as the significantly greater recovery from the urine as compared to single sulfonamides, can be explained by a more complete absorption of mixtures and the greater ease of their renal elimination. These findings were confirmed in extensive absorption and excretion studies with sublethal dosages of sulfonamide combinations administered by the intraperitoneal and oral route. They proved that for any given sulfonamide the completeness of absorption and urinary elimination was, within limits, inversely proportional to the size of the dose used. This behavior remained unchanged for each drug if several different sulfonamides were administered simultaneously. In other words, *in mixtures of sulfonamides the body handles each compound as if it were present alone and in the amount contained in the combination.*

With regard to *in vitro* antibacterial activity, it was reported previously[1] that the sulfathiazole-sulfadiazine combination showed essentially an additive effect and that in some instances the combination was even more effective than either compound alone in equal concentration. Continuation of these experiments rendered identical results for a mixture of sulfadiazine and sulfamerazine, and a combination of all 3 compounds. However, it should be remembered that these observations were made in the test tube with equal sulfonamide concentrations, whereas

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14 Lehr, D., *Bull. New York Acad. Med.*, 1944, **20**, 424.

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16 Beyer, K. H., Peters, L., Patch, E. A., and Russo, H. F., *J. Pharmacol.*, 1944, **82**, 239.

the administration of equal dosages of the 3 sulfonamides and their combinations would result in greatly different drug concentrations in the living body. In accordance with the results of absorption-excretion studies, as exemplified in Fig. 1, sulfonamide mixtures give high and well sustained blood concentrations and should, therefore, prove at least of the same if not of higher antibacterial value when compared with the same dosage of any one of their individual components *in vivo*. Although this viewpoint is well supported by clinical experience,[2,3] it remains to be accurately investigated in therapeutic studies with experimental infections of laboratory animals.

Summary. 1. In continuation of experimental and clinical studies with mixtures of sulfonamides, the toxicity as well as the absorption and excretion of the combinations sulfadiazine-sulfathiazole, sulfadiazine-sulfamerazine, and sulfadiazine-sulfathiazole-sulfamerazine were investigated in albino rats.

2. These combinations of partial dosages proved significantly less toxic than any one of their separate constituents in equal or comparable total dosage. The mixture of 3 sulfonamides was less toxic than either combination of 2 drugs.

3. The low toxicity of sulfonamide mixtures was shown to be due to the prevention of renal obstruction, resulting from a pronounced diminution in the intratubular deposition of sulfonamide crystals.

4. Mixtures of sulfonamides were more completely absorbed and excreted than equal amounts of their individual constituents. Blood levels from mixtures were, therefore, distinctly higher than expected on the basis of mathematical computations from the values of single sulfonamides.

5. It was reasoned on the basis of these experimental studies that the use of a mixture containing 3 sulfonamides in human therapy would almost completely eliminate the possibility of concrement formation in the urinary tract at the routine dose level. Hence, it would also obviate the necessity for adjuvant alkali therapy.

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Surface Striations of *Euglena gracilis* Revealed by Electron Microscopy.

VINCENT GROUPÉ. (Introduced by Geoffrey Rake.)

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In the course of studying a variety of microorganisms with the aid of electron microscopy using the shadow casting technic of Williams and Wyckoff[1] and the replica technic of Hillier and Baker[2] a definite pattern of surface striations on the pellicle of *Euglena gracilis* was clearly revealed. The presence of such surface striations on this species suggests the possibility that many or all of the species of *Euglena* possess these markings in varying degree inasmuch as prominent surface striations have been described for other species of *Euglena (e.g., E. viridis, E. oxyrus,* and *E. spirogyra.)*[3]

Bacteria-free cultures of 4 physiologically different species of *Euglena gracilis,* obtained through the courtesy of Dr. George W. Kidder, were maintained by serial passage on

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2 Hillier, J., and Baker, R. F., *J. Bact.*, 1946, **52**, 411.

3 Kudo, R. R., *Handbook of Protozoology,* Charles C. Thomas, Springfield, Ill., 1931, 117-120.