

LONDON SATURDAY DECEMBER 13 1947

PREVENTION OF RENAL DAMAGE BY USE OF MIXTURES OF SULPHONAMIDES*

ANIMAL-EXPERIMENTAL AND CLINICAL STUDIES

BY

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A new and simple approach to the prevention of concretions in the urinary tract caused by sulphonamides emerged from the observation that a saturated aqueous or urinary solution of one derivative of sulphanilamide could still be fully saturated with a second and third sulphonamide even if only of slightly different molecular structure, each of the compounds behaving as though it were present alone and exerting no influence on the solubility of the others. Consequently, in solutions containing several sulphonamides the maximum obtainable concentration appeared expressed by the additive solubilities of all individual drugs present (Lehr, 1945). This principle is illustrated in diagrammatic form in Fig. 1.

therapeutically equivalent sulphonamides rather than the full dosage of any one single compound. Actually the hazard of precipitation from such combinations should be only as great as if each sulphonamide had been administered alone and in the partial dosage contained in the mixture

Results of Animal Experimental Studies

Comparative studies of the toxicity and of the absorption and excretion of sulphonamide mixtures and their individual constituents were carried out on 750 albino rats from our own standard colony (Lehr, 1945, 1947). A condensation of the most important findings is presented in the accompanying tables and figures.

1. Acute Toxicity

Table I illustrates the values for the acute toxicity. It demonstrates that combinations of sulphonamides are significantly less toxic than comparable or equal amounts of their individual constituents. Estimation on the basis of the toxicity

TABLE I.—Acute Toxicity of Sulphonamides given Singly and in Various Combinations. (Single Intraperitoneal Injection of Sodium Salts in Albino Rats)

Drug	Dose (g./kg.)		No. of Animals	Deaths
	Partial	Total		
Sulphadiazine	1.5	1.5	90	85%
Sulphathiazole	1.1	1.1	25	65%
Sulphamerazine	1.5	1.5	29	52%
Sulphapyridine	1.1	1.1	35	63%
Sulphapyrazine	1.3	1.3	17	77%
Sulphadiazine	0.75	1.3	60	12%
Sulphathiazole	0.55			
Sulphadiazine	0.75	1.5	30	37%
Sulphamerazine	0.75			
Sulphadiazine	0.75	1.3	16	0
Sulphapyridine	0.55			
Sulphadiazine	0.54	1.5	40	0
Sulphathiazole	0.42			
Sulphamerazine	0.54			
Sulphadiazine	0.54	1.5	10	0
Sulphapyridine	0.42			
Sulphamerazine	0.54			
Sulphadiazine	0.54	1.5	10	0
Sulphapyrazine	0.42			
Sulphamerazine	0.54			

figures of the separate compounds would give, for example, an expected toxicity of about 77% for the sulphathiazole-sulphadiazine mixture, about 68% for the sulphamerazine-sulphadiazine combination, and about 74% for the mixture of sulphadiazine and sulphapyridine, whereas the actual values derived from experimental results were only 12%, 37%, and 0, respectively.

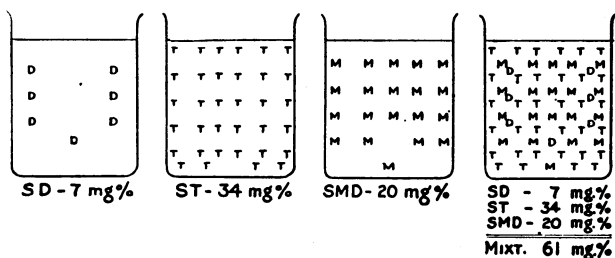


FIG. 1.—Diagram illustrating the individual and combined solubilities of sulphadiazine (SD), sulphathiazole (ST), and sulphamerazine (SMD) in water at room temperature (20° C.).

The figure elucidates the theoretical explanation of the phenomenon—namely, the specifically different molecular distribution in space of chemically different compounds. It also makes it clear that precipitation due to oversaturation will not occur more readily in the solution containing three sulphonamides, with a total concentration of 61 mg. per 100 ml., than in any one of the three individual solutions, with necessarily much lower maximum sulphonamide levels. Hence, at equal total sulphonamide concentrations the danger of precipitation will be significantly smaller in solutions of sulphonamide mixtures; it follows, in addition, that any increase in the number of components comprising a sulphonamide mixture will further diminish the chances of precipitation.

It was reasoned on the basis of this observation that the danger of the formation of sulphonamide crystals in the renal tubules could be considerably reduced by employing combinations of partial dosages of two or three

*Read at the Seventeenth International Physiological Congress in Oxford, on July 23, 1947.

†This investigation has been aided by grants from the Josiah Macy Jr. Foundation, New York City, and the Schering Corporation, Bloomfield, N.J.

Mixtures of *three* sulphonamides proved completely non-toxic at the 1.5 g. per kg. total dose level. This remarkably low toxicity pointed to a further increase in the protection afforded to the kidney against blockage with sulphonamide crystals.

Evidence of the presence or absence of renal obstruction was obtained from post-mortem examinations of the kidneys. Various degrees of kidney block caused by deposits of sulphonamide crystals in the renal tubules were observed in all animals succumbing to treatment with any of the five sulphonamides given singly. The kidneys appeared greatly enlarged and oedematous, and contained crystalline plugs in the papillary ducts, which in severe cases fanned out into the cortex. These drug deposits were clearly visible to the naked eye and easily demonstrable under the microscope. Rats treated with mixtures of three sulphonamides showed complete absence of renal blockage, and hence had normal kidneys. The lack of renal obstruction could also be inferred from normal non-protein nitrogen levels in the blood and high urinary elimination figures of sulphonamide. Added proof was derived from accurate chemical determinations of the sulphonamide amounts in the kidneys at various intervals after intraperitoneal injection of single and mixed sulphonamides.

Fig. 2 summarizes one representative experiment with animal groups killed 24 hours after intraperitoneal injection of sulphadiazine, sulphathiazole, sulphamerazine, and their combination

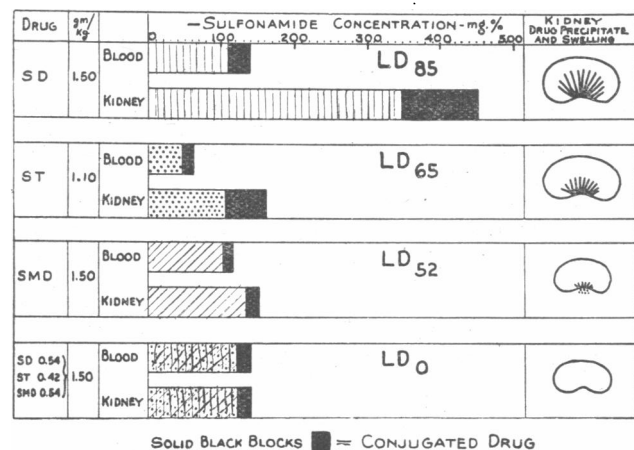


FIG. 2.—Findings in blood and kidneys of albino rats 24 hours after intraperitoneal injection of sulphadiazine (SD), sulphathiazole (ST), and sulphamerazine (SMD), given singly and in combination.

in the dosages used in the acute toxicity study. It indicates that rats injected with sulphadiazine showed the greatest discrepancy between blood and renal concentration of sulphonamide. The excessive concentration of sulphadiazine in the kidney is readily explainable on the basis of the massive crystalline deposition observed in this organ. The findings were similar although less marked in the sulphathiazole group. However, in this instance the large amount of conjugated sulphathiazole in the kidneys was of particular importance since the acetylated product has a solubility of only 7 mg. per 100 ml. at body temperature. Sulphamerazine caused the least intratubular precipitation, but showed the greatest tendency to the formation of gravel and concrements in the renal pelvis and ureters.

The mixture of all three sulphonamides did not produce any concrement formation in the urinary tract, and hence also did not reveal any difference between the concentration of sulphonamide in the blood and in the kidneys. It seems highly significant that the blood level of free sulphonamide resulting from the non-toxic dose of the mixture was actually higher than the levels obtained with comparable though fatal dosages of any one of the three mixture components under the conditions of renal impairment.

2. Subacute and Chronic Toxicity

The results of studies on the subacute toxicity are summarized in Table II. The daily dose of sulphonamides used was 0.9 g. per kg. In the mixture groups it consisted of equal amounts of the two or three compounds. The mortality figures shown

TABLE II.—Subacute Toxicity of Sulphonamides given Singly and in Various Combinations. (Intraperitoneal Injection of Sodium Salts for 5 Consecutive Days in Albino Rats)

Drug	Dose (g./kg.)		No of Animals	Deaths
	Partial	Total		
Sulphadiazine		0.9	25	100%
Sulphathiazole		0.9	15	100%
Sulphamerazine		0.9	20	75%
Sulphapyrazine		0.9	10	100%
Sulphapyridine		0.9	15	94%
Sulphadiazine	0.45 } 0.45 }	0.9	10	40%
Sulphathiazole				
Sulphadiazine	0.45 } 0.45 }	0.9	10	40%
Sulphamerazine				
Sulphadiazine	0.3 } 0.3 } 0.3 }	0.9	10	0
Sulphamerazine				
Sulphathiazole				
Sulphadiazine	0.3 } 0.3 } 0.3 }	0.9	10	0
Sulphamerazine				
Sulphapyridine				
Sulphadiazine	0.3 } 0.3 } 0.3 }	0.9	10	0
Sulphamerazine				
Sulphapyrazine				

are those observed on the sixth day of the experiment. The strikingly low toxicity of combinations as compared with the high mortality from separate compounds is immediately apparent. It should be mentioned, for the sake of completeness, that studies were also carried out with various mixtures containing *sulphacetamide* (N,-acetylsulphanilamide), a sulphonamide of relatively high solubility. They proved, as expected, that the toxicity of such combinations was particularly low.

The results of the acute and subacute studies were confirmed in part also in chronic feeding experiments with weaning albino rats, although in tests based on voluntary drug intake the results are of necessity less clear-cut. On the one hand the taste of the standard food seems to be spoiled by certain sulphonamides (sulphathiazole, sulphamerazine) and not by others (sulphadiazine), which results in great differences in the food intake. On the other hand, tolerable amounts ingested over a long period of time may be insufficient to produce fatal renal complications but high enough to result in serious reactions due to the tissue toxicity of the sulphonamides. Hence, for comparative investigations of the renal toxicity of sulphonamides and their mixtures the incorporation of the drugs into standard food for *voluntary* consumption is not a good procedure.

3. Absorption-Excretion

The comparative absorption from the peritoneal cavity is exemplified for three combinations with the dosages used in the evaluation of acute toxicity. Fig. 3 shows that the blood levels from the sulphathiazole-sulphadiazine mixture are at all times almost as high as from sulphadiazine alone, despite the low toxicity of this combination. The urine volumes indicate an unimpeded flow of urine in the rats treated with the mixture; this group consequently also reveals by far the highest values for urinary drug excretion.

In a similar manner Fig. 4 demonstrates that the blood levels and particularly the urine concentrations of the sulphamerazine-sulphadiazine combination are higher than expected on the basis of the values from the individual compounds. It is evident, in addition, from the columns representing total urinary elimination of the drugs (in this instance on a cumulative basis) that excretion of the mixture is far more complete than from either compound administered singly in equal amounts.

Fig. 5 illustrates the absorption and excretion of the mixture of *three* compounds. If compared with the previous two figures it is apparent that this sulphonamide combination gives excellent blood and urine concentrations. The total urinary elimination of sulphonamides is not only higher than from any one of the three compounds administered *singly*, but also significantly more complete than from a mixture of two. The same behaviour of sulphonamide mixtures was observed also with small dosages and from other routes of administration.

The fate of sulphonamides and their combinations after *oral* administration of *sublethal* amounts is depicted in a representative experiment with the sodium salts of sulphadiazine and

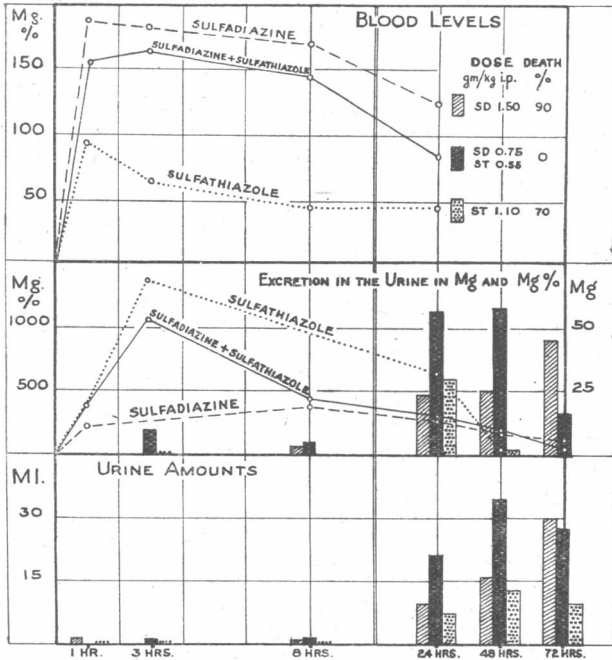


FIG. 3.—The absorption and excretion of sulphadiazine, sulphathiazole, and their combination in albino rats after intraperitoneal injection of single dosages. All figures represent the mean of the values of 10 animals.

sulphathiazole. Fig. 6 illustrates the absorption and excretion after intubation of 1 g. of the mixture per kg. of body weight as compared with the same weight amounts of the separate compounds. It is apparent from the graph that the mixture gives the highest blood levels, initially also the highest urine levels, and again by far the most complete urinary excretion, indicating that a combination of half dosages is more fully utilized than the same amount of either sulphadiazine or sulphathiazole.

The explanation for the more complete absorption and elimination of sulphonamide combinations emerged from tests

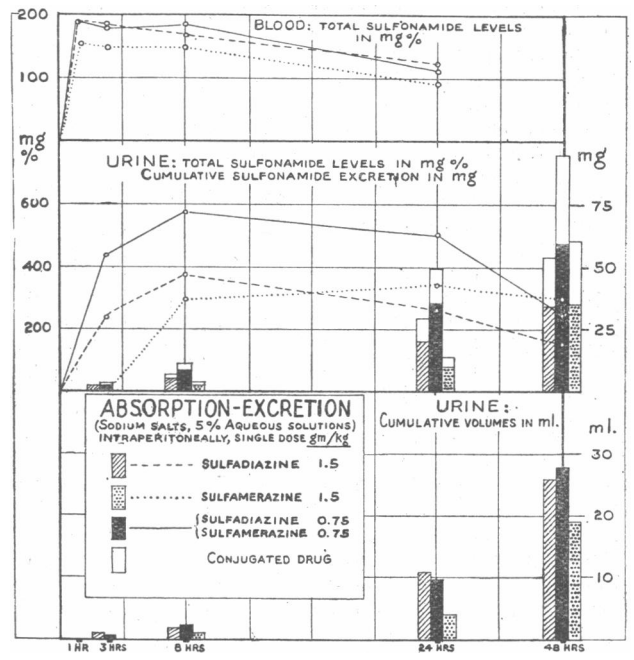


FIG. 4.—The absorption and excretion of sulphadiazine, sulphamerazine, and their combination in albino rats after intraperitoneal injection of single dosages. All figures represent the mean of the values of 10 animals.

with single drugs in the partial dosages contained in the mixture. These studies proved that smaller amounts are more completely absorbed from the gut, and consequently also more completely eliminated by the kidney. This behaviour remained apparently unchanged even if two sulphonamides were administered simultaneously. Hence, with regard to absorption and excretion, the body seems to handle a combination of sulphonamides as if each of its constituents were present alone and in the partial dosage contained in the mixture.

Table III illustrates this general principle in the renal excretion figures for the sulphathiazole-sulphadiazine mixture as

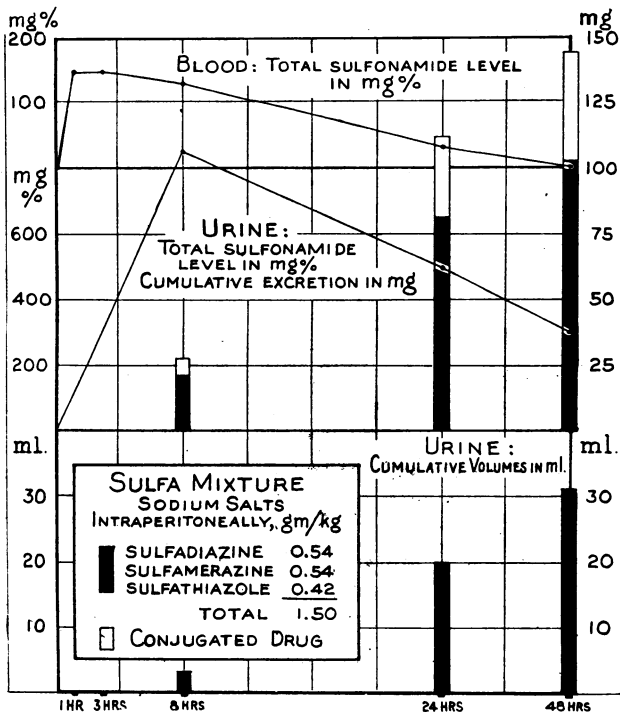


FIG. 5.—The absorption and excretion of a mixture containing partial dosages of sulphadiazine, sulphamerazine, and sulphathiazole after intraperitoneal injection of a single dose. All figures represent the mean of the values of 10 animals.

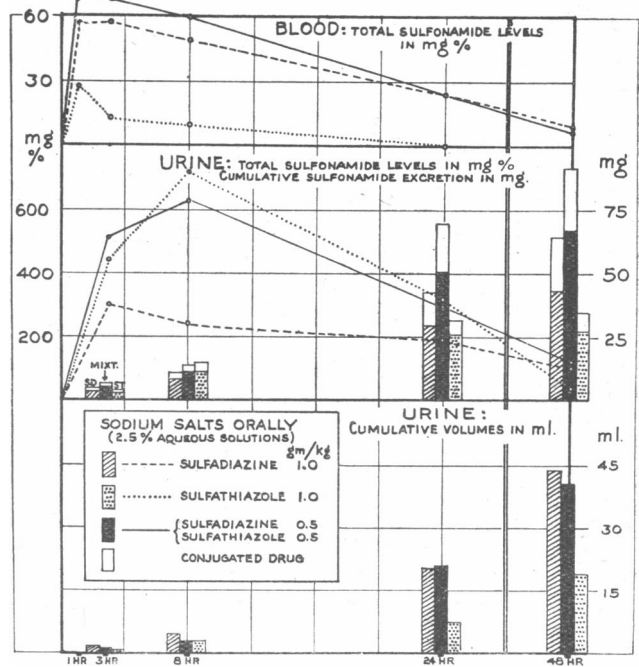


FIG. 6.—The absorption and excretion of sulphadiazine, sulphathiazole, and their combination after a single oral intubation. All figures represent the mean of the values of 10 animals.

contrasted with the values for the single compounds in equal and half dosages (Fig. 6). It is clearly apparent that the percentage of urinary excretion is strikingly higher from half

TABLE III.—*The Renal Elimination after 24 Hours of Sulphadiazine and Sulphathiazole, given Singly and in Combination. (Administration of Sodium Salts by Stomach Tube to Groups of 10 Albino Rats)*

Drug	Amount in g./kg. Body Weight		Amount of Dose Excreted
	Administered	Excreted	
Sulphadiazine	0.5	0.210	42%
	1.0	0.270	27%
Sulphathiazole	0.5	0.175	35%
	1.0	0.200	20%
Sulphadiazine Sulphathiazole	0.5 } 1.0 0.5 }	0.440	44%

dosages, whether given singly or in combinations. Incidentally, the observations in the laboratory animal that sulphonamide mixtures give good therapeutic blood and urine levels were fully confirmed in absorption-excretion studies in man.

Results of In-vitro Antibacterial Activity of Sulphonamide Mixtures

The main result of *in-vitro* experiments on antibacterial activity of sulphonamide mixtures was the observation that the action of similarly effective sulphonamides in combinations corresponds largely to the *total* content of sulphonamide; in other words, in mixtures of different but similarly effective sulphonamides the bacteriostatic effect is generally additive. True potentiation of action was observed in some instances (Lehr, 1945).

It should be kept in mind, however, that these results were obtained with equal sulphonamide concentrations in the test-tube, whereas the administration of equal dosages of the different sulphonamides and their combinations would result in significant differences in the drug concentrations in blood and tissues. In accordance with the results of the absorption-excretion studies, mixtures give excellent tissue levels; hence they should prove *in vivo* at least of the same, if not of higher, antibacterial value if compared with equal dosages of any one of their individual components. This point of view was well supported by *clinical results*.

Results of the Therapeutic Use of Sulphonamide Combinations

Up to the present a total of more than 700 unselected patients with systemic infections have been treated at the Flower and Fifth Avenue Hospitals and at the Metropolitan Hospital in New York City with a mixture containing equal amounts of sulphathiazole and sulphadiazine, or sulphadiazine and sulphamerazine, on a routine dosage schedule (Lehr, 1946; Lehr *et al.*, 1946). The oral route was used in the majority of cases. In children the sulphadiazine-sulphamerazine combination was also given by the subcutaneous route. These results will be published later. Adequate fluid intake was assured, but no alkalinizing agents were employed.

The therapeutic response to both sulphonamide combinations was highly satisfactory in most instances. Defervescence and general clinical improvement seemed to occur with greater speed when compared with previous experience with any of the drugs administered separately. Hence the total amount of sulphonamide used was comparatively small. Therapeutically effective blood levels (5 to 20 mg. free sulphonamide per 100 ml.) were maintained with ease. Urinary concentrations of sulphonamide varied between 100 and 600 mg. per 100 ml. Conjugation figures were as a rule low, ranging between 3 and 20 mg.

per 100 ml. in the blood, and 10 and 40 mg. per 100 ml. in the urine.

Crystalluria was infrequent, and never of the "massive" type observed so commonly during therapy with single compounds. Of more than 900 acid morning specimens of urine containing sulphonamide mixtures which were examined under the microscope after sharp centrifugation only about 7% were found to hold moderate or small amounts of sulphonamide crystals, as contrasted with the incidence of crystalluria of 29% from sulphadiazine, 26% from sulphamerazine, and 70% from sulphathiazole alone (Flippin *et al.*, 1941; Flippin and Reinhold, 1946). None of the patients treated with mixtures developed any sign of serious renal irritation. Quite unexpectedly the incidence of allergic reactions also seemed to have decreased. Nausea and vomiting were rare. No other toxic reactions were encountered. At present we are engaged in clinical studies with various mixtures of three sulphonamides.

Confirmation of these investigations came recently from several sources. Whitehead (1946), at the University of Colorado, confirmed the animal experimental data. Flippin and Reinhold (1946), at the University of Pennsylvania, extended the clinical observations. They showed in a well-controlled study that "the use of sulphadiazine and sulphamerazine in mixtures containing equal parts of each drug led to a considerably decreased incidence of crystalluria compared with that observed when either compound was administered singly." They concluded that for the prevention of crystalluria the use of the mixture was as effective as the administration of sodium bicarbonate at the rate of 12 g. a day with either sulphadiazine or sulphamerazine. Finally, Hagerman and his co-workers (Frisk *et al.*, 1946, 1947), in Sweden, using a combination of sulphathiazole, sulphadiazine, and sulphamerazine, arrived independently at confirmatory animal-experimental and clinical results.

Summary

A new and simple approach to the prevention of renal complications from sulphonamide therapy is presented. It consists in the use of a *mixture* of several sulphonamides in partial dosages instead of single compounds. In solution each sulphonamide component of the mixture behaves as if present alone, exerting no influence on the solubility of the others. Consequently the danger of drug precipitation in the kidney was significantly decreased with sulphonamide mixtures, as shown in animal experimental and clinical studies. Actually it was only as great as if each compound had been given alone and in the partial dosage contained in the combination. On the other hand the chemotherapeutic effect of these mixtures was roughly proportional to their total sulphonamide content.

The results of these studies indicate that combinations of two or more sulphonamides should be used in preference to single compounds for all indications, since they combine a high therapeutic efficacy with a remarkably low renal toxicity.

The principle of dissolving several structurally similar and therapeutically equivalent drugs in the same medium to the full extent of the separate saturation levels for each compound might prove of value in any instance where sparingly soluble drugs have to be incorporated into small volumes of solvent.

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