fore, presented separately. Somewhat different results were also obtained with the 2 series of mice which received glucose or starch by stomach tube.

As may be seen from the table, only starch and glucose acted as did the mixed food in increasing the proportion of mice showing injured lungs, and glucose had this effect whether it was given intragastrically or intraperitoneally. Just as in the mice that were previously treated with oxygen, the action of olive oil seemed to protect against injury, rather than sensitize to it.

Summary. Mice that had been permitted access to a mixed food before exposure to high tensions of oxygen with a subsequent sudden fall of total pressure to 70 mm were found much more likely to succumb to such treatment than were mice that had been deprived of food for a day. This effect of food was also observed after the administration of glucose, intragastrically or intraperitoneally, but not after the intragastric administration of olive oil.

Mice not previously treated with high tensions of oxygen were nearly all killed by the sudden exposure to 70 mm pressure, but those that had been deprived of food did not show the marked congestion of the lungs that was observed in mice that had been fed a mixed food. Mice that had received glucose or starch showed this congestion, but those that had received xylose, olive oil, egg albumin, leucine, or sodium chloride did not.

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Studies on Rates of Hydrolysis of N^4 Dibasic Acid Substituted Sulfonamides.*

EDGAR J. POTH AND CHARLES A. ROSS.

From the Surgical Research Laboratory, Department of Surgery, The University of Texas Medical Branch, Galveston, Texas.

The reports of Poth, Knotts, Lee and Inui[1] and Poth and Ross[2] detailed the results of a study of 33 sulfonamides which were examined for bacteriostatic activity in the gastrointestinal tract. Fourteen of these compounds were N^4 acyl (dibasic acid) derivatives. Succinylsulfathiazole and phthalylsulfathiazole, of this series, have been shown to possess considerable local bacteriostasis in the gastrointestinal tract and were studied extensively both experimentally and clinically.[3-11]

Many of the other members of this series were found to have little antibacterial activity in the bowel. Since the conjugated sulfonamides have but slight bacteriostatic activity, the simplest explanation of the activity of these compounds should be due to the free sulfonamides liberated by the hydrolysis of the acylated compounds. If this were true, then the rates of hydrolysis of these sulfonamides in aqueous solutions of varying hydrogen ion concentrations should furnish an index to their antibacterial activity.

Poth, Knotts, Lee, and Inui[1] observed that

* Supported by a grant from Sharp & Dohme, Inc., Philadelphia, Penn.

1 Poth, E. J., Knotts, F. L., Lee, J. T., and Inui, F., *Arch. Surg.*, 1942, **44**, 187.

2 Poth, E. J., and Ross, C. A., *Tex. Rep. Biol. Med.*, 1943, **1**, 345.

3 Poth, E. J., Chenoweth, B. M., Jr., and Knotts, F. L., *J. Lab. and Clin. Med.*, 1942, **28**, 162.

4 Smyth, C. J., Gould, S. E., and Finkelstein, M. B., *J. A. M. A.*, 1943, **121**, 1244.

5 Hardy, A. V., Burns, W., and DeCapito, T., *Pub. Health Rep.*, 1943, **58**, 689.

6 Twyman, A. H., and Horton, G. R., *J. A. M. A.*, 1943, **123**, 138.

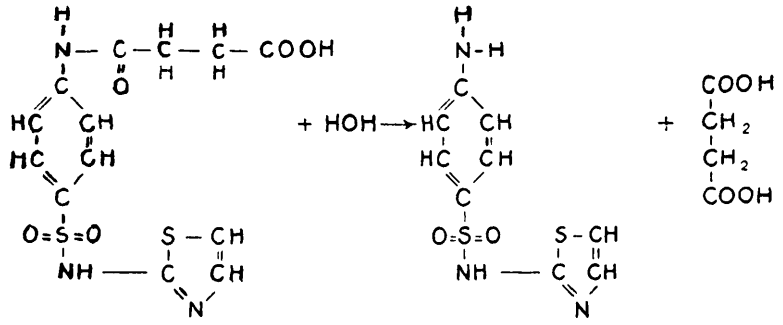
7 Poth, E. J., *J. A. M. A.*, 1942, **120**, 265.

8 Behrend, M., *S. Clin. North America*, 1944, **24**, 238.

9 Crohn, B. B., *Gastroenterology*, 1943, **1**, 140.

10 Bloomfield, A. L., and Lew, W., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 28.

11 Menchaca, F., *An. Soc. puericult.*, Buenos Aires, 1942, **8**, 399.



Succinylsulfathiazole *Sulfathiazole* *Succinic Acid*

those compounds which were easily hydrolyzed to the free aryl amine, were readily absorbed, and were usually more toxic than the relatively stable derivatives. It was observed by Poth and Ross[12] that a much higher concentration of free sulfathiazole was obtained in feces with phthalyl derivative of sulfathiazole than with the succinyl derivative when equal doses of the drugs were administered. This observation may be taken to explain why phthalylsulfathiazole is twice as effective bacteriostatically as succinylsulfathiazole. While the concentration of free sulfonamide in the feces may not account for the total bacteriostatic effect, it would seem that it may be related in some manner to the activity of members of this series of compounds.

Method. Millimolar solutions of the N^4 acyl sulfonamides are made in 1.0 N-HCl and 1.0 N-NaOH and maintained at 38°C. Samples are withdrawn at the desired time intervals and quantitative estimations of the concentration of the free aryl amine made using a modification of the colorimetric procedure of Bratton and Marshall.[13] An Evelyn colorimeter is employed.

Rates of Hydrolysis of the Conjugated Sulfonamides. When the conjugated compounds in solution are treated with acid or alkali, the compounds are split to yield a molecule of the free aryl amine, or free sulfonamide, and a dibasic acid: *i.e.*,

If the N^4 substituent is a saturated dibasic organic acid, the hydrolysis rates are more rapid in alkaline than in acid solution as shown in Charts I and II and Table I-A. First order equations do not fit these hydrolysis curves. If, however, the N^4 substituent is the unsaturated organic acid, maleic acid, then the speeds of the hydrolysis rates in alkali and acid are reversed (See Table I-B). Likewise, if the N^4 substituents are the aromatic organic acids, phthalic and quinolinic acids, the rates

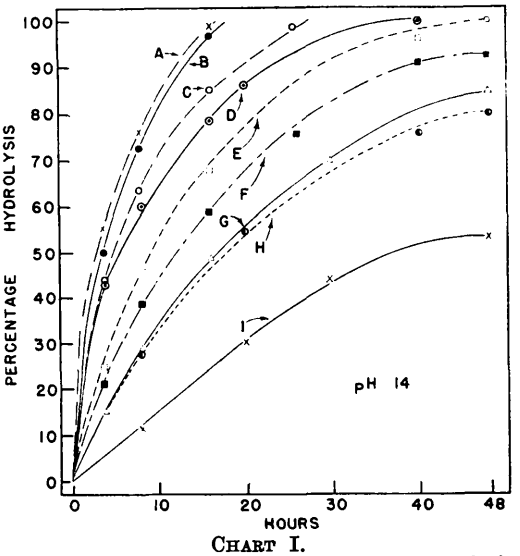


CHART I.

Graphic representation of the rates of hydrolysis of N^4 aliphatic dibasic acid substituted sulfonamides in 1.0 N-NaOH at 38°C.

A. Succinylsulfaguanidine
B. Oxalylsulfathiazole
C. Succinylsulfapyridine
D. Succinylsulfathiazole
E. Succinylsulfanilamide
F. Maleylsulfathiazole
G. Malonylsulfathiazole
H. Succinylsulfamethyldiazine
I. Maleylsulfanilamide

Each compound is present in one millimolar concentration.

[12] Poth, E. J., and Ross, C. A., *J. Lab. and Clin. Med.*, 1944, **29**, 785.

[13] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **125**, 537.

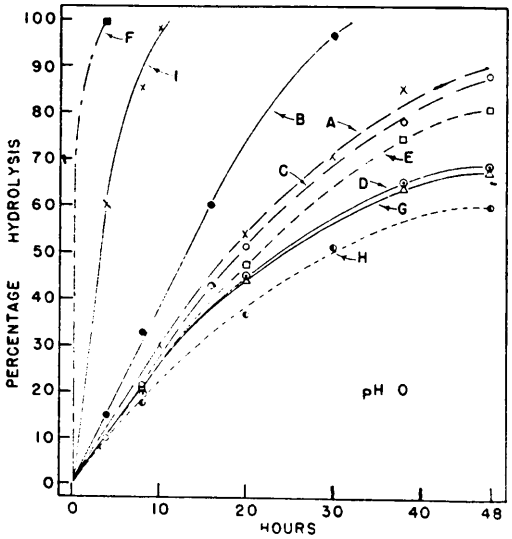


CHART II.

Graphic representation of the rates of hydrolysis of N^4 aliphatic dibasic acid substituted sulfonamides in 1.0 N-HCl at 38°C.

A. Succinylsulfaguanidine
B. Oxalylsulfathiazole
C. Succinylsulfapyridine
D. Succinylsulfathiazole
E. Succinylsulfanilamide
F. Maleylsulfathiazole
G. Malonylsulfathiazole
H. Succinylsulfamethyldiazine
I. Maleylsulfanilamide

Each compound is present in one millimolar concentration.

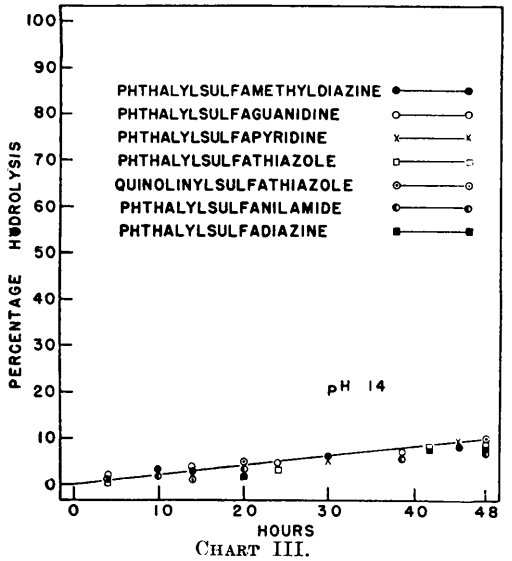


CHART III.

Graphic representation of the rates of hydrolysis of N^4 aromatic dibasic acid substituted sulfonamides in 1.0 N-NaOH at 38°C. Each compound is present in one millimolar concentration.

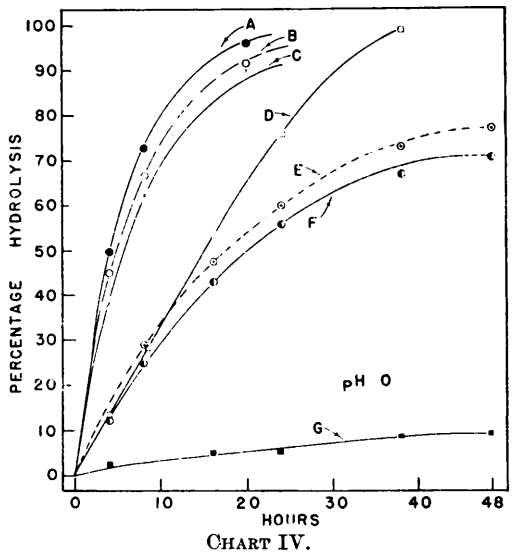


CHART IV.

Graphic representation of the rates of hydrolysis of N^4 aromatic dibasic acid substituted sulfonamides in 1.0 N-HCl at 38°C.

A. Phthalylsulfamethyldiazine
B. Phthalylsulfaguanidine
C. Phthalylsulfapyridine
D. Phthalylsulfathiazole
E. Quinolinylsulfathiazole
F. Phthalylsulfanilamide
G. Phthalylsulfadiazine

Each compound is present in one millimolar concentration.

of hydrolysis in acid are relatively rapid while the compounds are quite stable in alkaline solutions (Table I-C).

Considering succinylsulfathiazole, a representative of the saturated substituents, and phthalylsulfathiazole, a representative of the aromatic substituents, Chart V, it is evident that in the region of pH 7, the hydrolysis rate for each is slowest. (See also charts 7 and 8, pp. 365-6, Poth and Ross.[2])

Correlation of Hydrolysis Rate to Bacteriostatic Activity. If the antibacterial activity of these acylated sulfonamides is due to the free sulfonamide resulting from hydrolysis or deacylation, then the bacteriostatic activity of these compounds should be directly related to the tendency for deacylation to occur, Table I. This relationship should be even more evident in an homologous series of compounds such as is indicated in Table II. It will be noted, however, that such is not always true. The hydrolysis rates of succinylsulfathiazole and malonylsulfathiazole are essentially the same,

TABLE I.

Comparison of Rates of Hydrolysis, Bacteriostatic Activity, Concentration of Drug in Blood, and Toxicity of Various Conjugated Sulfonamides Following Oral Administration to the Dog.

The compounds are listed in 3 groups: *Group A*, the acylating radical is a saturated, dibasic organic acid; *Group B*, the acylating radical is an unsaturated, aliphatic, dibasic, organic acid; and *Group C*, the acylating radical is an aromatic, dibasic, organic acid. The rates of hydrolysis are determined in 1.0 N-HCl and 1.0 N-NaOH at 37°C. The bacteriostatic activity is the effect observed on the coliform organisms in the bowel of the dog following the oral administration of 0.167 g of drug per kilo of body weight every 4 hours. The toxicity and concentration of the drug in the blood are recorded for the same dosage level.

Conjugated sulfonamide	Relative rate of hydrolysis in normal		Relative bacteriostatic activity	Concentration of sulfonamide in mg per 100 cc of blood		Relative toxicity
	HCl	NaOH		Free	Conjugated	
Group A						
Succinylsulfaguanidine	++±	+++±	++±	—	—	0
Succinylsulfapyridine	++±	+++	+++	—	33.0	+++
Succinylsulfathiazole	++	+++	++++	3.5	5.0	0
Succinylsulfanilamide	++±	++±	++++	2.5	1.5	0
Succinylsulfamethyldiazine	+	++	++	—	—	0
Oxalylsulfathiazole	+++	+++±	+	—	—	+++
Malonylsulfathiazole	++	++±	±0	—	—	+++
Group B						
Maleylsulfathiazole	++++	++±	++++	10.95*	—	0
Maleylsulfanilamide	++++	+	++++	2.0	2.0	++++
Group C						
Phthalylsulfaguanidine	+++±	±	—	—	—	—
Phthalylsulfapyridine	+++±	±	—	—	—	—
Phthalylsulfamethyldiazine	+++±	±	0	4.3	5.0	0
Phthalylsulfathiazole	+++	±	+++++	3.0	3.0	0
Phthalylsulfanilamide	++	±	+	1.5	1.0	0
Phthalylsulfadiazine	±	±	0	4.3	1.0	0
Quinolinylsulfathiazole	++	±	++++	—	—	0

* Maleylsulfathiazole is so susceptible to acid hydrolysis that it is completely split in the course of the analytical procedure, which makes it impossible to determine the distribution of free and conjugated compounds in the blood.

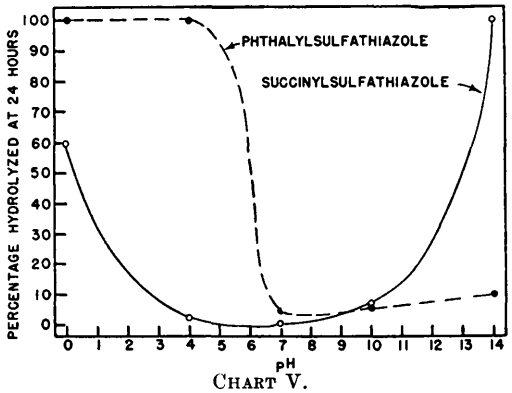


CHART V.

Comparison of the hydrolysis rates of succinylsulfathiazole and phthalylsulfathiazole at different hydrogen ion concentrations varying between that of 1.0 N-HCl and 1.0 N-NaOH at 38°C. The drugs are present in a concentration of 0.05 millimolar.

but succinylsulfathiazole is strongly bacteriostatic against the coliform organisms in the bowel of the dog while malonylsulfathiazole has only slight activity.

Likewise, the rate of hydrolysis of oxalylsulfathiazole in both acid and base greatly exceeds that of succinylsulfathiazole, and again the bacteriostatic action of the former is but a fraction of the latter compound.

The rate of hydrolysis in acid solution frequently parallels the bacteriostatic activity, but the rate of hydrolysis in basic solution gives no indication of the bactericidal action even in an homologous series of compounds. This latter fact is particularly emphasized by a comparison of quinolinylsulfathiazole, phthalylsulfathiazole, succinylsulfathiazole, and malonylsulfathiazole, Table II.

In the final analysis, of course, the rates of deacylation at the pH of the medium in which the drugs must act in the host is really the important consideration. In this connection the succinyl and phthalyl derivatives were incubated at 38°C with a suspension of human feces adjusted to pH 7, Table III. In this

TABLE II.

Comparison of Hydrolysis Rates and Bacteriostatic Activity of an Homologous Series of Acylated Sulfathiazole Derivatives.

These data are compiled from the same sources as those of Table I and were obtained under the same experimental conditions.

Acylated sulfathiazoles	Relative hydrolysis rates in normal HCl	Relative hydrolysis rates in normal NaOH	Relative bacteriostatic activity
Succinylsulfathiazole	++	+++	++++
Oxalylsulfathiazole	+++	+++±	+
Malonylsulfathiazole	++	++±	+
Maleylsulfathiazole	++++	++±	++++
Phthalylsulfathiazole	+++	±	+++++
Quinolinylsulfathiazole	++	±	++++

TABLE III.

Rate of Hydrolysis of Acylated Sulfonamides in a Suspension of Human Feces.

0.2 millimoles of the respective compounds are mixed with 100 cc of a suspension containing 5.0 g of wet feces. The mixture is adjusted to pH 7 using a phosphate buffer and is incubated at 38°C. The initial coliform count is 110,000 per cc, which increased to approximately 10,000,000 per cc within the first 24 hours in each instance.

Acylated sulfonamide	Hours	Conc. of free sulfonamide in mg per 100 cc 0	24	48	72	Initial conc. of conjugated drug in mg per 100 cc
Succinylsulfathiazole		1.3	5.6	6.9	11.9	71.0
Succinylsulfanilamide		1.4	4.3	5.7	10.0	54.6
Phthalylsulfathiazole		14.5*	15.8	15.6	13.1	80.6
Phthalylsulfanilamide		7.4*	6.7	7.4	6.9	64.0
Phthalylsulfadiazine		14.4*	12.5	15.0	15.0	79.8
Phthalylsulfamethyldiazine		16.8*	14.7	16.9	15.3	81.4
Phthalylsulfathiazole in buffer only		12.8*	13.5	14.0	13.5	80.6

* The relatively high values of free sulfonamide in these determinations are inherent in the analytical procedure, due to the rapidity with which the phthalyl derivatives are hydrolyzed in acid solution. Note that these values do not increase with incubation of the suspensions.

heterogeneous mixture of feces, containing many bacteria and various enzymes and ferments, succinylsulfanilamide and succinylsulfathiazole are appreciably hydrolyzed while the phthalyl derivatives show no tendency to be split. The finding that the phthalyl derivatives are chemically more stable is not to be expected from previous observations which show phthalylsulfathiazole to be more readily deacylated to maintain a higher concentration of free sulfonamide in the bowel than in the case of succinylsulfathiazole.

Discussion. An examination of the hydrolysis rates at which this series of conjugated sulfonamides undergo deacylation fails to indicate always the magnitude of the local, antibacterial activity of any single, individual, compound. These same facts do, however, again add some evidence to suggest that the entire antibacterial activity of these conjugated sulfonamides is not due solely to the action of free sulfonamides produced by the hydrolysis of the acylated derivative. From other observations,[12] we have postulated that the local bacteriostatic action of this series of compounds may arise from several possible sources: (1) the activity may be derived from the free sulfonamide liberated by hydrolysis;[1,14] (2) a portion of the activity may result from the physical and chemical properties of the conjugated compound which determine the local concentration of the unaltered drug adsorbed onto or absorbed into the individual susceptible microörganism, where; (3) the conjugated compound is acted upon by ferments within the cell to yield highly reactive split-products in a nascent or excited state, which products would in their turn enter

[14] Kirby, W. M. M., and Rantz, L. A., *J. A. M. A.*, 1942, **19**, 615.

into competitive reactions to prevent the formation of essential metabolites from specific substrates; and, (4) the activity may be influenced by the formation of split products not containing the sulfonamide radical such as succinate from succinyl derivatives. Since the antibacterial activity of members of an homologous series of these compounds does not parallel the ease with which sulfathiazole, for example, is formed by simple hydrolysis of the succinyl, phthalyl, quinolinyl, malonyl, oxalyl, and maleyl derivatives of sulfathiazole, it seems highly probable that the biologic activity is not explained entirely if it be assumed that such activity is due entirely to the sulfathiazole present.

Similarly, the high toxicity of maleylsulfanilamide as compared to the relatively low toxicity of maleylsulfathiazole cannot be explained upon the assumption that it is due to the split products, because sulfanilamide does not possess any such high degree of toxicity when compared to sulfathiazole.

Conclusion. Much of the data presented here cannot be correlated to show a relationship between antibacterial activity and rate of deacylation of the conjugated compounds, and it seems clear that the antibacterial activity of these compounds is not due solely to the liberation of the free sulfonamides. A considerable part of the antibacterial activity of these substances must, therefore, reside in the conjugated molecule. The node of action is complex and cannot be defined at this time.

The toxicity of the various compounds, likewise, does not necessarily parallel the chemical instability of the several conjugated sulfonamides.

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Effect of Steroid Substances on Synthesis of Acetylcholine.*

CLARA TORDA AND HAROLD G. WOLFF.

From the New York Hospital, and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York, N.Y.

Previous investigations have shown that male and female sex hormones may exert a vasomotor effect on the small vessels;[1-17] hormones of the adrenal cortex may change the size of the vessels due to changes induced in water and ion metabolism of the body;[18-20] estrogenic substances may increase the amount

* This study was aided by a grant from the Josiah Macy, Jr., Foundation.

1 Carloni, E., *Arch. di ostet. e ginec.*, 1930, **17**, 327.

2 Markee, J. E., *Am. J. Physiol.*, 1932, **100**, 32.

3 Valle, G., *Ann. di ostet. e ginec.*, 1934, **56**, 1011.

4 Mortimer, H., Wright, R. P., Bachman, C., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 535.

5 Reynolds, S. R. M., *Am. J. Obstet. and Gynec.*, 1938, **36**, 437.

6 Reynolds, S. R. M., and Foster, F. I., *Am. J. Physiol.*, 1939, **128**, 147.

7 Reynolds, S. R. M., and Foster, F. I., *J. Clin. Invest.*, 1939, **18**, 649.

8 Reynolds, S. R. M., and Foster, F. I., *J. Pharm. Exp. Therap.*, 1940, **68**, 173.

9 Reynolds, S. R. M., *J. Invest. Dermatology*, 1940, **4**, 7.

10 Hamblen, E. C., Cuyler, W. K., and Hirst, D. V., *Endocrinology*, 1940, **27**, 172.

11 Reynolds, S. R. M., Kaminester, S., Foster, F. I., and Schloss, S., *Am. J. Obstet. and Gynec.*, 1941, **41**, 1022.

12 Edwards, E. A., Hamilton, J. B., Duntley, S. Q., and Hubert, G., *Endocrinology*, 1941, **28**, 119.

13 Reynolds, S. R. M., Kaminester, S., Foster, F. I., and Schloss, S., *Surg., Gynec., and Obstet.*, 1941, **73**, 206.

14 Hirst, D. V., and Hamblen, E. C., *J. Clin. Endocrinology*, 1942, **2**, 664.

15 Reynolds, S. R. M., Hamilton, J. B., di Palma, J. R., Hubert, G. R., and Foster, F. I., *J. Clin. Endocrinology*, 1942, **2**, 228.

16 Hirst, D. V., Hamblen, E. C., and Cuyler, W. K., *J. Clin. Endocrinology*, 1942, **2**, 442.

17 Walker, J. C., *J. Clin. Endocrinol.*, 1942, **2**, 560.