SUMMARY.

1. That the efficiency of the mastic-talc coating was found to be 48.5 per cent as compared with 62.5 per cent for mastic-magnesium stearate.

2. That the efficiency of the mastic-talc coating was not sufficiently high to warrant its recommendation for use on enteric medicaments. However, it must be remembered that this material has a higher efficiency value than such material as shellac, shellac-salol, which are in common commercial use.

3. That the efficiency of mastic-magnesium stearate as an enteric coating material was sufficiently high to warrant its recommendation to the pharmaceutical manufacturers.

4. That both the mastic-talc and the mastic-magnesium stearate coating were applicable to factory methods in which the coating pan is used.

5. That a high-boiling solvent such as methyl propyl ketone (B. P. 102° C.) was a better solvent for the mastic than the more volatile solvents, such as acetone (B. P. 56.5° C.) since the higher boiling solvent did not produce blisters in the coating.

6. That it is possible to determine the entirety of an enteric coating from the weight of the enteric material adhering to the pill, capsule or tablet, assuming they were uniform in size. The determination was based on the average gain in weight for lots of twenty-five or more pills, capsules or tablets.

7. That for a better degree of enteric efficiency, it was found advisable to make this determination from the number of pills disintegrating in the small intestine and the ascending colon, since absorption from the transverse and descending colons would not be as complete.

SULFANILAMIDE ADDITION COMPOUNDS WITH CINCHONA-ALKALOIDS.*

BY E. H. STUART, H. M. POWELL, C. L. ROSE AND F. E. BIBBINS.*

In 1935 Domagk (1) announced that certain azo dye derivatives were specific remedies in the treatment of infections by beta hemolytic streptococcus. Out of this work came *p*-aminobenzenesulfonamide, officially named by the Council on Pharmacy and Chemistry of the American Medical Association, "Sulfanilamide" (2). This compound has proven to be one of the outstanding developments in the field of chemotherapy during recent years. There has been an increasing amount of evidence with regard to the extraordinary effectiveness of sulfanilamide against various infections with the result that it is now reported as having been tried in the treatment of a great variety of diseases, including malaria.

The chemotherapeutic action of the sulfanilamide compounds on malaria reported in the literature are both favorable and unfavorable although the total number of favorable case reports far exceed the unfavorable ones. Hill and Goodwin, Jr., (3) reported on the use of 2,4-di-aminoazobenzene-4′-sulfonamide (4) in one hundred cases of plasmodium vivax malaria with beneficial results. Read and Pino (5) used 2,4-diaminoazobenzene-4′-sulfonamide in three cases of plasmodium

* From the Control Laboratories, Eli Lilly and Company.

vivax malaria with negative results. Van der Wielen (6) used 2,4-diaminoazobenzene-4′-sulfonamide in two cases of quartan malaria with favorable results. Faget (7) reported unfavorably on four cases (two of plasmodium vivax, one case of plasmodium falciparum and one case of plasmodium malariæ) treated with sulfanilamide and 2,4-d aminoazobenzene-4′-sulfonamide. He stated that quinine had to be administered before recovery. Pakenham-Walsh, Oxon and Rennie (8) reported good results on benign tertian malaria in one case treated with 2,4-diaminoazobenzene-4′-sulfonamide. Hall (9) treated four cases of tertian malaria with 2,4-diaminoazobenzene-4′-sulfonamide and sulfanilamide (4) with unfavorable results.

In 1937 de Leon (10) treated fifteen cases of benign tertian fever with success using 2,4-diaminoazobenzene-4′-sulfonamide. In 1938 de Leon (11) reported again on the advantages of sulfanilamide in the treatment of malaria, in which he stressed the fact that sufficient doses of sulfanilamide must be used in order to get good results. Coggeshall (12) stated that sulfanilamide was found to have a prophylactic value and a marked therapeutic effect on acute plasmodium knowlesi infections in rhesus monkeys. Chopra and Das Gupta (13) found that disodium *p* (γ-phenylpropylamino) benzenesulfonamide α-γ-disulfonate and 2,4-diaminoazobenzene-4′-sulfonamide were effective for plasmodium knowlesi infection in rhesus monkeys.

We have recently found that sulfanilamide will form compound salts with the cinchona-alkaloids similar to quinine and urea hydrochloride. These compounds are homogeneous crystalline salts of the cinchona-alkaloid and sulfanilamide in molecular proportions. They have definite physical and chemical properties. For instance, they form definite crystals, and those crystals usually have definite melting points. The sulfanilamide component cannot be separated from the cinchona-alkaloid component of the crystals by extraction with acetone, as is the case of mixtures of sulfanilamide and quinine salts. The crystals of most of the compounds are yellow in color, although a few are white, whereas both the sulfanilamide and the common cinchona-alkaloid salts are white in color. The crystals are homogeneously soluble in various solvents and usually have a solubility which is different both from that of the sulfanilamide and from that of the cinchona-alkaloid salt.

In order to prepare a cinchona-alkaloid sulfanilamide salt it is necessary that there be a sufficient amount of a strong mineral acid present, such as one of the halogen acids, a sulfonic acid or sulfuric acid, to satisfy the acid-combining power of the cinchona-alkaloid component. That is, assuming that hydrochloric acid (a monobasic acid) is the acid, there must be at least two molecules of hydrochloric acid, or if sulfuric acid (a dibasic acid) is the acid, there must be at least one molecule of sulfuric acid for each molecule of the cinchona-alkaloid component. More acid than the amount named above may be present in the reaction, and in the case of quinine and quinidine, but apparently not in the case of cichonine and cinchonidine, the crystals which are obtained in the presence of the excess acid may contain a higher percentage of acid, apparently enough more to satisfy the acid-combining power of the sulfanilamide component also.

PREPARATION OF QUININE SULFANILAMIDE H_2SO_4.

Add to 30 cc. distilled water 4.2 Gm. of quinine bisulfate and 1.7 Gm. of sulfanilamide. Mix thoroughly and heat until all the material is in solution. The crystals form on cooling somewhat below room temperature. They may be easily recrystallized by dissolving in about 30 cc. boiling distilled water and cooling.

Analysis shows that the quinine sulfanilamide bisulfate made by the above method contains quinine and sulfanilamide in substantially molecular proportions associated with one molecule of sulfuric acid. The crystals are white and melt at 208° C., corrected. They have a specific optical rotation using a 2.5 per cent solution in water of $[\alpha]_D^{25°}$ C. = − 147°.

PREPARATION OF QUININE SULFANILAMIDE $1^1/_2$ H_2SO_4.

This compound is similar to the preceding example except that instead of using 30 cc. of water at the outset 5 cc. is used containing 0.5 cc. of concentrated sulfuric acid, and the compound is recrystallized from 5 cc. of water rather than from 30 cc. Analysis shows that these crystals contain quinine and sulfanilamide in substantially molecular proportions, associated with one and one-half molecules of sulfuric acid. The compound is white and has a melting point of 186° C., corrected; and a specific optical rotation, as observed in a 2.5 per cent solution in water of $[\alpha]_D^{25°}$ C. = − 112°.

In the preparation of the above compounds alcohol may be used as the solvent in place of water.

By using a similar method the compounds indicated in the following table have been prepared:

TABLE I.—CINCHONA-ALKALOID SULFANILAMIDE COMPOUNDS.

Compounds.	M. P.	$[\alpha]_D^{25°}$ C.	Color of Crystals
Quinine Sulfanilamide 2 HCl (1 mol. water of cryst.)	110° C.	−150°	Yellow
Quinine Sulfanilamide 2HCl (Anhydrous)	130° C.	−155°	Yellow
Quinine Sulfanilamide 3HCl	150° C.	−144°	Light yellow
Quinine Sulfanilamide 2HBr	211° C.	−126°	Light yellow
Quinine Sulfanilamide 2HI	about 70° C.	−92.5°	Yellow
Quinine Sulfanilamide H_2SO_4	208° C.	−147°	White
Quinine Sulfanilamide $1^1/_2$ H_2SO_4	186° C.	−112°	White
Quinine Sulfanilamide Sulfamic Acid	133° C.	−131°	Light yellow
Quinine Sulfanilamide Sulfanilyl Sulfanilic Acid	153° C.	...	White
Quinidine Sulfanilamide 2HCl	about 135° C.	+170°	Yellow
Quinidine Sulfanilamide 2HBr	about 130° C.	+137.5°	Yellow
Quinidine Sulfanilamide H_2SO_4	172° C.	+164°	Light yellow
Quinidine Sulfanilamide $1^1/_2$ H_2SO_4	125° C.	+152°	Yellow
Euquinine Sulfanilamide 2HCl	about 135° C.	−55°	Yellow
Euquinine Sulfanilamide 2HBr	about 135° C.	−43°	Yellow
Euquinine Sulfanilamide H_2SO_4	91° C.	−51.5°	Yellow
Cinchonine Sulfanilamide 2HCl	about 135° C.	+132°	Yellow
Cinchonine Sulfanilamide 2HBr	about 130° C.	+112°	Yellow
Cinchonine Sulfanilamide H_2SO_4	about 120° C.	+123.5°	Yellow
Cinchonidine Sulfanilamide 2HCl	about 136° C.	−93.7°	Yellow
Cinchonidine Sulfanilamide 2HBr	about 135° C.	−78°	Yellow
Cinchonidine Sulfanilamide H_2SO_4	180° C.	−92.5°	Cream

TABLE I.—CINCHONA-ALKALOID SULFANILAMIDE COMPOUNDS. (*Continued from page 92.*)

Compounds.	M. P.	$[\alpha]_D^{25°}$ C.	Color of Crystals
Quinine 4, 4 Diaminodiphenyl Sulphone H_2SO_4	176° C.	...	Yellow
Quinine Disulfanilamide 2 HCl (14)	115° C.	...	Yellow
Quinine Disulfanilamide H_2SO_4	207° C.	−145°	White
Quinine N^1 Hydroxysulfanilamide H_2SO_4 [a,b]	187° C.	...	White
Quinine *P*-benzylaminobenzenesulfonamide H_2SO_4 [b]	104° C.	...	Light yellow
Quinine *P*-mandelylaminobenzenesulfonamide H_2SO_4 [b]	190° C.	...	White
Quinine 2 (*p*-aminobenzenesulfonamide) pyridine H_2SO_4 [b]	139° C.	...	Light yellow

[a] The system of naming as given in *J. American Chemical Society*, Vol. 60, page 2217.

[b] It is of interest to note that in the case of these compounds the sulfanilamide has been substituted on either the amino or the amide portion of the molecule without preventing the formation of the compounds with the cinchona-alkaloids.

In the few cases where the optical rotation is not given the compounds were not sufficiently soluble in water to make this determination.

ACTION OF CERTAIN OF THESE COMPOUNDS ON MICE INFECTED INTRAPERITONEALLY WITH HEMOLYTIC STREPTOCOCCUS.

Sixteen hour serum broth culture of streptococcus "Todd" was used to make serial decimal dilutions in chilled broth. Within one-half hour nine test mice, in groups of three, received doses of 10^{-2}, 10^{-3} and 10^{-4} cc., respectively, while control mice in pairs received doses of 10^{-2}, to 10^{-9} cc. of the streptococci thus prepared intraperitoneally (see Table II). The various groups of test mice received drug treatment as indicated.

Quinine sulfanilamide 3 HCl and sulfanilamide seem to be equally efficient chemotherapeutically as shown in Table II. The remaining compounds were less effective against streptococcal infection, but nearly all showed some degree of effectiveness.

ACTION OF THESE COMPOUNDS ON MICE INFECTED INTRAPERITONEALLY WITH STAPHYLOCOCCI COATED WITH MUCIN TO ENHANCE VIRULENCE.

The following compounds in doses indicated, have also been subjected to standardized mouse therapeutic tests against staphylococcal infected mice, mucin coated staphylococci being used for establishing infection.

All compounds were tested on six mice, three mice receiving 1000 M. L. D. and three receiving 10,000 M. L. D. of culture. The therapy comprised two subcutaneous doses given one hour and three hours after infection.

1. Quinine 4,4-diaminodiphenyl sulfane bisulfate—5 mg.
2. Quinine sulfanamide N'Hydroxysulfanilamide sulfate—5 mg.
3. Quinine sulfanilamide camphorsulfanate—0.5 mg.

None of these compounds showed any efficacy toward staphylococcal infection in mice inasmuch as test mice and control mice died after the same period of time.

ACTION OF THESE COMPOUNDS ON MICE INFECTED WITH INFLUENZA VIRUS.

The following compounds, in doses indicated have been subjected to standardized mouse therapeutic tests against influenza virus. These compounds were tested in groups of eight mice, two mice being infected intranasally with virus dilutions 1–10, 1–100, 1–1000 and 1–10,000, respectively, with the exception of compound No. 4 which was tested on six mice, three mice being infected with virus dilutions 1–10 and 1–100, respectively. Untreated virulence controls of the virus were included. All the mice used in these tests were Swiss mice. Four doses of drug were given subcutaneously to the different groups of test mice, one dose being given on the same day as the virus and one dose on each of the next three days.

1. Quinine sulfanilamide 2HCl—5 mg.
2. Quinine sulfanamide N'Hydroxysulfanilamide sulfate—5 mg.
3. Quinine 4,4-diaminodiphenyl sulfone bisulfate—5 mg.
4. Quinine sulfanilamide camphorsulfonate—0.5 mg.

None of the above compounds showed any efficacy against influenza virus, inasmuch as treated test mice died along with untreated controls, or on the weaker dilutions of virus the treated and untreated mice showed the same degree of pulmonary lesions upon autopsy at the end of the standard 14-day observation period.

TABLE II.—CURATIVE TEST OF STREPTOCOCCUS-INFECTED MICE.

Compounds.	Drug* Dose in Mg.	Dose of Streptococcus "Todd" in Cc. 10^{-3}.	10^{-4}.	10^{-5}.	10^{-6}.	10^{-7}.
Quinine Sulfanilamide 2 HCl	5	SSS	SSD	SSD		
Quinine Sulfanilamide 3 HCl	5	SSD	SSS	SSS		
Quinine Sulfanilamide H_2SO_4	5	SDD	DDD	SSS		
Quinine Sulfanilamide Camphorsulfonate	0.5	DDD	SDD	SDD		
Cinchonidine Sulfanilamide H_2SO_4	5	SDD	SDD	SSS		
Cinchonine Sulfanilamide H_2SO_4	5	SSD	SSD	SSD		
Quinidine Sulfanilamide H_2SO_4	5	SDD	SSS	SDD		
Euquinine Sulfanilamide H_2SO_4	5	SSD	SSD	SSD		
Quinine Disulfanilamide 2 HCl	5	SSD	SSS	SSD		
Quinine 4, 4-Diaminodiphenyl Sulfone H_2SO_4	5	SSD	SSS	SSD		
Sulfanilamide	5	DSS	SSS	SSS		
Untreated Control	..	DD	DD	DD	DD	SS

S—mouse surviving D—mouse died of infection Duration of test—14 days

* The drug dose indicated was administered subcutaneously one hour and five hours after infection.

TABLE III.—TOXICITY OF CERTAIN OF THESE COMPOUNDS BY INTRAVENOUS INJECTION IN MICE.

Substance.	Mg./Kg.	No. Mice Died.	No. Mice Used.
Euquinine Sulfanilamide Bisulfate	50	0	3
	70	0	3
	80	3	3
Quinidine Sulfanilamide Bisulfate	120	1	4
	130	3	5
	140	3	4
Cinchonidine Sulfanilamide Bisulfate	150	2	5
	160	3	4
	200	3	3
Quinine Sulfanilamide Bisulfate	160	0	3
	170	3	5
	180	3	3
Cinchonine Sulfanilamide Bisulfate	220	1	4
	230	3	4
	250	3	4

THE ACTION OF THE CINCHONINE SULFANILAMIDE BISULFATE AGAINST PLASMODIUM RELICTUM IN CANARIES.

Cinchonine sulfanilamide bisulfate, the least toxic of this group, was tested according to the method of Tate and Vincent (15). These authors reported 2.5 mg. of quinine hydrochloride per 20 Gm. as being the minimal effective dose and 10 mg. per 20 Gm. of the canaries weight, the

maximal tolerated dose. We have used, therefore, doses of cinchonine sulfanilamide bisulfate approximately equivalent to 10 and 2.5 mg. of quinine hydrochloride per 20 Gm. of the canary body weight. In addition one dose equivalent to 2 mg. per 20 Gm. of the bird's weight has been administered. None of these doses produced sterility in the canaries and although the largest dose caused some apparent lag in the rate of appearance of the parasites in the blood stream of the canaries, it was no more than might be expected by treating the birds with cinchonine alone.

THE ACTION OF QUININE SULFANILAMIDE DIHYDROCHLORIDE AGAINST PLASMODIUM RELICTUM IN CANARIES.

Coggeshall (12) in a recent report showed that sulfanilamide alone was ineffective against plasmodium cathemerium infections in canaries or plasmodium lophuræ infections in young chicks.

We have tried, in addition to cinchonine sulfanilamide bisulfate, quinine sulfanilamide dihydrochloride as well as sulfanilamide alone against plasmodium relictum in canaries. In the case of sulfanilamide, there was no response, thus confirming in part Coggeshall's findings. With the use of quinine sulfanilamide dihydrochloride there was a definite lag in the rate of appearance of the parasites as compared with untreated but innoculated control birds, this lag, as in the preceding case, seemed to be in proportion to the amount of quinine given, rather than to the quantity of whole drug.

SUMMARY.

1. The preparation of sulfanilamide addition compounds with cinchona-alkaloids has been described.

2. Several of these new compounds have indicated promising chemotherapeutic activity against experimental hemolytic streptococcus infections when given in proportion to their sulfanilamide content.

3. None of the new compounds tested have shown indications of effectiveness in chemotherapy of staphylococcus infections or of human influenza virus infections in Swiss mice.

4. Those of the new compounds tested in chemotherapy of malaria infected canaries have been active only in proportion to their cinchona-alkaloid content.

BIBLIOGRAPHY.

(1) Domagk, G., *Deutsche Med. Wchnschr.*, 61, 250 (1935).

(2) Council on Pharmacy and Chemistry, *J. A. M. A.*, 108, 1340 (1937).

(3) Hill, R. A., and Goodwin, M. H., Jr., *Southern Med. J.*, 30, 1170–1172 (Dec. 1937).

(4) "Sulfanilamide Therapy of Bacterial Infections," by Ralph R. Mellon, pub. by Charles C. Thomas, Baltimore, Md., Pages 6, 10 and 11.

(5) Read, H., and Pino, O., *Arch. F. Schilfs U. Tropen-Hyg.*, 42, 132 (1938).

(6) Van der Wielen, Y., *Nederl. Tijdschr. V. Geneesk*, 81, 2905 (1937).

(7) Faget, G. H., *Public Health Reports*, 53, 1364–1366 (Aug. 5, 1938).

(8) Pakenham-Walsh, Oxon and Rennie, *Lancet*, 2, 79 (July 9, 1938).

(9) Hall, W. E. B., *J. Pharmacology and Experimental Therapeutics*, 63, 353–356 (Aug. 1938).

(10) de Leon, A. D., *Public Health Reports*, 52, 1460–1462 (October 15, 1937).

(11) de Leon, A. D., *Medicina*, 18, 471–473 (Sept. 25, 1938).

(12) Coggeshall, L. T., *Proceeding of the Society for Experimental Biology and Medicine*, 38, 768 (June 1938).

(13) Chopra, R. N., and Das Gupta, B. M., *Indian M. Gaz.*, 73, 395–396, and 665–667 (1938).

(14) Rosenthal, S. M., *Public Health Reports*, 52, 662 (1937), *Ibid.*, 53, 40 (1938).

(15) Tate, P., and Vincent, M., *Parasitology*, 25, 411–427 (1933).