64 to about 16,000, the anti-B from 32 to 8000. Further investigations on a larger scale are necessary in order to determine the conditions which will produce maximum rise in antibody titer of donors treated with purified A and B substances. The experiment recorded in Table II suggests that minute amounts of A and B substances are able to stimulate a powerful antibody response.

All the injections described in this report have been given intravenously. To what extent subcutaneous, intradermal, or intramuscular injections can replace the intravenous administration is under investigation.‡

The important points regarding quality of a test serum are titer, speed, and specificity. The sera of donors treated with A and B substances excel by their speedy agglutination and produce complete agglutination of cells within a short time on slides and in test tubes. Even A_2 cells which are characterized by a relatively weak agglutinability are usually strongly agglutinated in a short time. We have had no opportunity so far to examine A_3 cells. The specificity of the test sera obtained by treatment with A and B substances was in all instances satisfactory.

Conclusions. The intravenous treatment of donors with purified A and B specific substances isolated from animal sources leads to a considerable increase in isoantibody titer. The substances used were highly purified and did not contain any demonstrable hog or horse protein. The administration of even small amounts is effective and results in the production of sera which are characterized by high titer, speed and specificity. The procedure described easily permits the production of large quantities of very potent typing serum.

‡ In one recent case of Group O the intramuscular injection of 1 cc of a stock solution of AB specific substances resulted in a 100-fold increase in both isoagglutinins Anti-A and Anti-B.

14505

Chemotherapy of Infections with *Cl perfringens* in Mice by Homosulfonamides.*

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The homosulfonamides[1,2] are not inhibited by *p*-amino benzoic acid.[3] Disappointing results with the sulfonamides in the treatment of clinical gas gangrene[4] due, in part at least, as far as their local use is concerned, possibly to the *p*-amino benzoic acid present in wounds, led us to investigate the toxicity and the efficacy of the homosulfonamides in experimental gas gangrene in mice. Reports on the use of homosulfanilamide (*p*-(α-amino)-toluene sulfonamide hydrochloride) have been inconclusive, owing partly to the variety of technics and the small numbers of animals used to test this compound.[5,6,7,8,9] It has been shown that homosulfanilamide was bacteriostatic *in vitro* for *Cl. perfringens* and for

* Homosulfanilamide has been described in the German literature under the name of Marfanil.

1 Miller, E., Sprague, J. M., Kissinger, L. W., and McBurney, L. F., *J. Am. Chem. Soc.*, 1940, **62**, 2099.

2 Klarer, J., *Klin. Woch.*, 1941, **20**, 1250.

3 Schreus, H. T., *Klin. Woch.*, 1942, **21**, 671.

4 MacLennan, J. D., *Lancet*, 1943, **265**, 123.

5 Schreus, H. T., and Peltzer, E., *Klin. Woch.*, 1941, **20**, 504.

6 Schreus, H. T., Brauns, A., and Schümmer, H., *Klin. Woch.*, 1941, **20**, 1233.

7 Schreus, H. T., *Klin. Woch.*, 1942, **21**, 14.

8 Domagk, G., *Klin. Woch.*, 1942, **21**, 448.

9 Klöse, F., and Schröber, W., *Zent. f. Bakt.*, I Abt. orig., 1942, **149**, 15.

TABLE I.
Acute Toxicity of Homosulfonamides.

Drug	Solution injected %	Solution injected pH	Dosage, g/kg	mM/kg	No. of mice	% mortality	Calculated LD_{50} and S.E. of LD_{50} as mM/kg
132			3.0	13.5	25	0	
	8.0	4.3	4.0	18.0	45	42	18.5 ± 0.5
	10.0	4.5	5.0	22.5	25	88	
684	2.0	5.0	1.0	4.2	5	0	
	5.0	5.1	2.5	10.6	20	50	10.6*
			3.0	12.7	5	80	
			4.0	16.9	5	100	
627			1.0	3.3	5	0	
	2.4	4.0	1.2	3.9	20	25	
	2.8	4.1	1.4	4.6	20	65	4.37 ± 0.03
	3.2	3.9	1.6	5.2	20	90	
	4.0	3.9	2.0	6.6	5	100	
			3.0	9.9	5	100	
617			1.34	4.6	10	90	
	3.08	5.6	1.54	5.2	10	90	< 4.6
			1.92	6.6	5	100	
690	12.86	5.8	6.43	22.5	20	5	>22.5

* Approximation.

a strain of sulfathiazole-fast *Cl. perfringens*,[10] and that bacteriostatic activity was not inhibited by *p*-amino benzoic acid.

The homosulfonamides, prepared in the Division of Medicinal Chemistry of the Squibb Institute,[11] were:

132—*p*-(α-amino)-toluene sulfonamide hydrochloride (homosulfanilamide)
617—*p*-[N-(4,6 dimethyl) pyrimidyl-2]-(α-amino)-toluene sulfonamide (homosulfamethazine)
627—*p*-(N-thiazyl-2) (α-amino)-toluene sulfonamide hydrochloride (homosulfathiazole)
654—*p*-(N-thiazyl-2)-(α-phthalimido)-toluene sulfonamide
684—*p*-(N-methyl) (α-amino)-toluene sulfonamide hydrochloride
690—*p*-(α-succinamido)-toluene sulfonamide
713—*p*-(α-sulfanilamido)-toluene sulfonamide

Toxicity Studies. The work was hampered by the lack of a satisfactory method of quantitatively determining these drugs. A comparison of the acute and chronic toxic effects of 132 with its succinamido and several of its N substituted derivatives was made. (Sufficient amounts of 654 and 713 were not available. A mortality of approximately 60% was caused in mice receiving acacia suspensions of 654 or 713 intraperitoneally in doses of 0.6 or 2.0 g per kg respectively.) The compounds were used as soluble hydrochlorides, except the succinamido derivative which was dissolved in dilute alkali. Swiss mice were injected intraperitoneally and observed for 72 hours. The acute toxicity experiments are summarized in Table I. With a few exceptions, all deaths occurred within 2 hours after administration; none were observed after 24 hours. On the basis of millimols of drug per kilogram of body weight, all the derivatives, with the exception of the succinamido, were distinctly more toxic than 132.

The effects of continued administration of 132 and sulfanilamide were studied in groups of 10 mice each. The mice were put on diets containing 1, 2, and 4% of the former as well as equivalent levels (0.775, 1.55, and 3.1%) of the latter for a period of 28 days. The weight curves of the 7 groups (Fig. 1) demonstrate little or no difference in chronic toxicity of the 2 drugs at the 2 lowest concentrations. However, at the highest level

[10] MeKee, C. M., Hamre, D. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 211.

[11] Braker, W., and Bergeim, F. H., to be published.

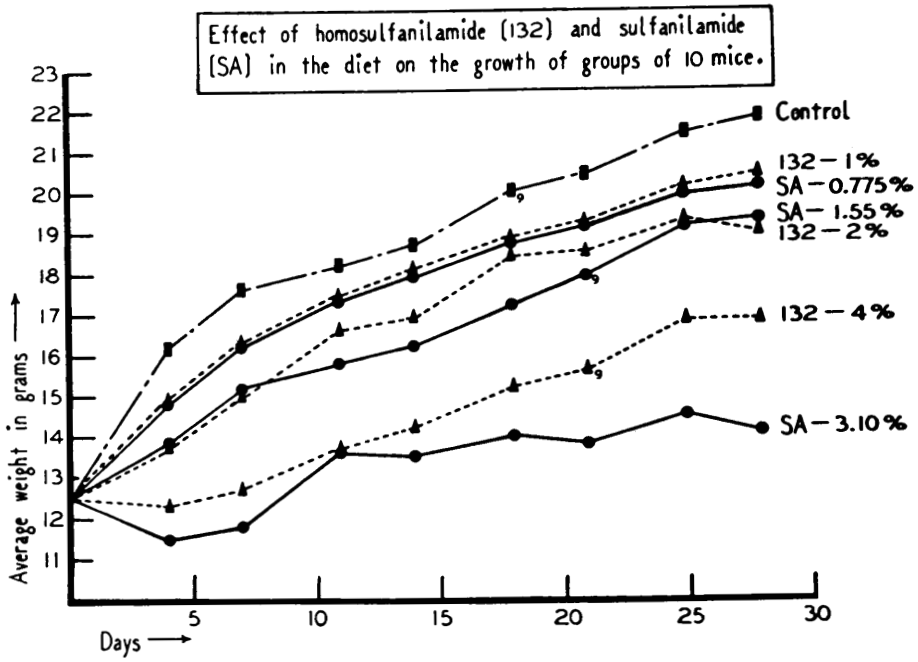

FIG. I

sulfanilamide had a distinctly greater effect than 132 on the growth of mice. One control mouse died on the 18th day of experiment, while on the 21st day one mouse each died on diets of 4% of 132 and 1.55% of sulfanilamide. On the 28th day the animals were sacrificed and the gross appearance of the organs of all mice was examined. The spleens of all mice receiving sulfanilamide were congested and slightly enlarged. Except for one mouse on the diet of 4% of 132, in which the spleen and kidneys were pale, no other gross changes were observed. Portions of the liver, kidneys, and spleen, of 4 mice on each diet were fixed for microscopic study. The only significant change in the livers of mice receiving 132 was a slight degree of fatty infiltration occurring on the highest drug level. All the mice receiving sulfanilamide were found to have hyperplastic spleens with large deposits of hemosiderin which also was unusually prominent in sections of the livers. One mouse on 4% homosulfanilamide had a marked hydronephrosis apparently owing to drug obstruction; a similar change, much less pronounced, was found in another mouse of this group. Tubular dilatation, apparently owing to mechanical obstruction, was also observed in the kidneys of 2 mice on the highest diet level of sulfanilamide.

Therapeutic Studies. Mice were treated immediately after intramuscular infection with *Cl. perfringens*[10] by injecting into the same site 0.1 ml of solutions of the homosulfonamides or sulfadiazine. They were observed for 3 weeks. It should be pointed out that this technic does not give entirely consistent results in repeated tests. In Table II are given all the results for compounds at the dose levels used in 3 or more tests. 654 and 713 were relatively insoluble, which in part accounts for the inconsistent results at the two dose levels of 713. In tests in which local treatment was delayed 1 hour, 132 still gave some protection.

At the dose levels employed, administered either in the diet or intraperitoneally (654—0.5% in diet, 684—500 mg/kg per day, 713—280 mg/kg per day or 0.14% in diet), 654, 684, and 713 did not protect mice infected intraperitoneally with *H. influenzæ* 62/B, *Strep. hemolyticus* C203, or *Staph. aureus* Smith. When given by intraperitoneal injection (500 mg/kg per day), 132 did not protect mice infected intraperitoneally with *Strep. hemolyticus* or *Staph. aureus*, while at

TABLE II.
Local Treatment of Mice Injected Intramuscularly with *Cl. perfringens.*

Drug	Mg inj.	No. of tests	No. of mice	No. lived	No. died	% lived
NaSD*	1.0	5	40	37	3	92
"	0.1	7	60	52	8	87
132	1.0	3	30	23	7	77
"	0.5	6	60	35	25	58
617	1.0	3	25	10	15	40
627	1.0	3	25	9	16	36
654†	1.0	3	30	12	18	40
684	1.0	4	40	28	12	70
"	0.5	5	50	32	18	64
690	1.0	3	30	10	20	33
713†	1.0	4	40	2	38	5
" †	0.5	3	30	15	15	50
Controls	—	17	150	6	144	4

* Sodium sulfadiazine.
† Suspended in acacia.

1% in the diet, a slight degree of protection against *Strep. hemolyticus,* but not against *Staph. aureus,* was obtained. 132 at 1% in the diet did not protect mice infected with *Cl. perfringens,* although 1% sulfathiazole protected 50%. That these results were not due to non-absorption of 132 was shown by detection of this drug in the urine by means of *in vitro* tests with *Cl. perfringens.*

Three of the compounds were tested for bacteriostatic action on 15 strains of staphylococci by a serial dilution test in charcoal-adsorbed beef heart infusion broth, containing very little *p*-amino benzoic acid.[12] At 6.2 mg % all the strains were inhibited by 132 and 684 for 10 hours, although some grew in 17 hours. 132 and 684 also prevented growth of all strains for 17 hours when present in a concentration of 25 mg %, although by 57 hours all strains had grown. 690 had no demonstrable action.

[12] MacLeod, C. M., and Mirick, G. S., *J. Bact.,* 1942, **44**, 277.

Summary. Except for sulfadiazine, homosulfanilamide and its N^1-methyl derivative were the most effective drugs in local treatment of experimental gas gangrene of the compounds tested. The acute toxicity of homosulfanilamide was less than that of its derivative. The chronic toxicity of homosulfanilamide in mice appeared to be no greater than that of sulfanilamide. These two compounds were not effective in the treatment of infections in mice with *Strep. hemolyticus* C203, *H. influenzæ* 62/B, or *Staph. aureus* Smith, but they were bacteriostatic *in vitro* for staphylococci. Although infection with *Cl. perfringens* in mice is not strictly comparable with infection in man, these results indicate that homosulfanilamide may be worthy of a clinical trial in the local treatment of gas gangrene.

14506

Differentiation Between Fievre Boutonneuse and Rocky Mountain Spotted Fever by Means of Complement Fixation.

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It has been shown by Badger[1] that Fievre Boutonneuse, a disease of the Mediterranean basin transmitted by *Rhipicephalus sanguineus* ticks, and Rocky Mountain spotted fever are related, for one disease protects

[1] Badger, L. F., *Public Health Rep.,* 1933, **48**, 507.