TABLE I.
Summary of Data on Immunization of Chickens with Meningococci.

Animals employed			Immunizing antigens			Type-specific serologic properties of antiserum	
No.	Breed	Sex	Serologic type	Strain	Age of culture (hr)	Capsular swelling	Agglutination titer
68	Rhode Island Red	♀	I	1027	16-18	+	120
1	Plymouth Rock	♂	I	1027	4	+	240
7	White Rock	"	I	1027	4	+	120
66	Rhode Island Red	♀	II	963	16-18	0	80
49	" " "	"	II α	1054	16-18	+	40
3	Buff Orpington	♂	II α	1054	4	+	160
8	White Rock	"	II α	1054	4	+	320

Types I, II and II alpha, as others have done.[2,3,5]

Investigation was then made to determine whether these agglutinating sera would also exhibit the property of capsular swelling as is the case with rabbit anti-meningococcal sera of adequate potency. Suspensions of living organisms from 4- or 5-hour cultures on Mueller-Hinton medium were mixed in the usual fashion with a loopful of chicken antiserum plus a loopful of methylene blue stain. Microscopic observation revealed prompt and strikingly clear-cut capsular swelling of meningococci Types I and II alpha in the presence of their homologous antisera; no capsular swelling was demonstrable when cocci of these serologic types were mixed with any heterologous antiserum. No capsular swelling was seen in the mixtures of Type II meningococci with the single homologous serum available or with the several antisera versus the other 2 types. The above findings have been checked and found to hold true not only for the various sera as freshly drawn but also after storage at 2°C for periods from 8 to 14 months. The data in Table I also indicate that sex or breed does not markedly affect the success of immunization providing healthy adult birds, weighing 5 to 7 pounds, are employed.

Discussion. So far as we are aware, data on the use of chickens for the preparation of specific capsular swelling antisera against meningococci or other bacterial species have not been previously reported. The present findings are of interest in demonstrating that capsular swelling may be produced with antisera derived from animals other than the rabbit and the horse; they also suggest that chickens can be used to prepare quellung sera for bacteria to which the rabbit or horse may not respond favorably.

From a practical standpoint, the ease with which adult chickens can be successfully immunized for the production of quellung antiserum versus meningococci Types I and II alpha should make it feasible for many diagnostic laboratories to prepare a supply. Such sera can undoubtedly be used for rapid and direct serologic identification of meningococci in the spinal fluid as well as in cultures obtained from other sources.

[5] Kabat, E. A., Miller, C. P., Kaiser, H., and Foster, A. Z., *J. Exp. Med.*, 1945, **81**, 1.

15369

The Activity of Anionic Surface Active Compounds in Producing Vascular Obliteration.

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The great variety of substances used for obliteration of varicose veins have the common property of being destructive to intima of the vein.[1,2] Some of the substances are

used in a strongly hypertonic concentration (glucose, sodium chloride) and dehydrate through osmosis; others form a highly alkaline solution which is incompatible with cell life even for a relatively short period of time (sodium carbonate, soap solutions); still others contain substances which are poisonous to the protoplasma of cells in general (quinine hydrochloride, halogens, mercuric chloride). Differences in the manner in which the intima of the vein is destroyed are suggested by the differences in the rate of thrombus formation after injection. Mercuric chloride produces thrombus in 3 or more days,[3] whereas complete obliteration takes place within 24 hours after the injection of 20% sodium salicylate solutions.[4] Even more pronounced are the effects produced by soap solutions.[1] As this may be owing at least in part to the surface activity of the fatty acid anions and the anionic micelles formed in aqueous solutions of soaps, it was of interest to study the effectiveness of the synthetic anionic detergents in obliterating veins. The results of this study are reported in the following.

Methods. The marginal ear vein of rabbits and the veins of dogs have been used frequently to study the thrombogenic and sclerosing properties of various agents.[5,6,7]

In preliminary experiments using the marginal ear vein of rabbits it was found that it is difficult to regulate the rate of dilution of the sclerosing solution with blood, which obviously is one of the important factors determining the extent of thrombus formation. Because of the great number of collaterals, dilution of the agent after injection was rapid and high concentrations of the agent had to be used. These destroyed the delicate marginal ear vein and its collaterals rapidly and completely, permitting free diffusion of the agent and producing inflammatory reactions and necrosis of the surrounding tissue. Even if thrombus was produced without much tissue irritation, it was often extended to collaterals and its length could not easily be measured.

Much better results were obtained when the tail vein of the mouse was injected. Here, again, high concentrations of the agents, 2% of sodium ricinoleate or the equivalent in sclerosing strength of the anionic synthetic detergents, caused gangrene within 24 to 48 hours and the entire tail sloughed off within a few days. However, if the injection was carried out at a very slow rate, thrombus could be produced using lower concentrations of most of the agents without much tissue irritation.

In order to obtain well reproducible averages it was necessary to standardize the procedure as follows: mice weighing 20 to 25 g were fasted for 20 hours before injection. They were placed in a holder which permitted free handling of the tail. The veins were distended by dipping the tail for 1 minute into water which was kept at a temperature of 45 C. The solution was injected from a 0.25 cc Tuberculin Syringe No. LT ¼ with divisions of 0.01 cc through a 26-gauge needle having a short bevel. The needle was inserted at a distance of 50 mm from the base of the tail so that the tip of the needle was about 45 mm from the base of the tail. Five-hundredths cc was injected over a period of 10 seconds. Dilution of the solution with blood was prevented for 50 seconds by compressing the vein at the base of the tail and at the site of the injection. The length of the thrombus was measured with a ruler. In the dose range used, thrombus was usually formed within 24 hours. Delayed thrombus formation was observed occasionally with the lowest concentrations used. The optimum time for reading giving the maximum average length of thrombus was at about 48 to 96 hours, although the differences in results obtained when the reading was made at any time

[1] Oehsner, A., and Mahorner, H., *Varicose Veins*, The C. V. Mosby Company, St. Louis, 1939.

[2] McPheeters, H. O., and Anderson, J., *Injection Treatment of Varicose Veins and Hemorrhoids*, F. A. Davis Company, Philadelphia, 1943.

[3] Régard, G. L., *Rev. med. de la Suisse Rom., Genevé*, 1925, **65**, 102.

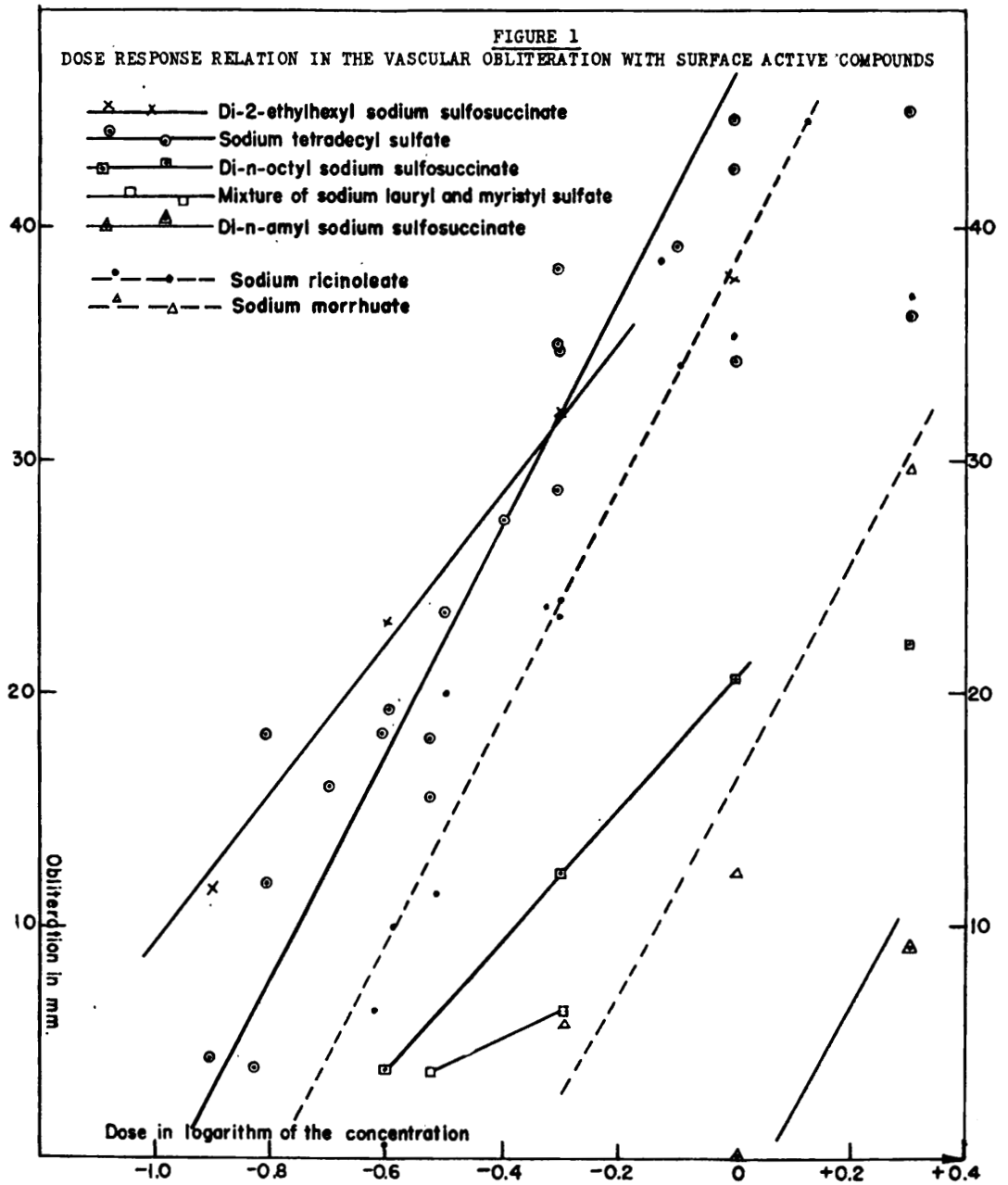
[4] von Meisen, *Acta chir. Scandinav., Stockholm*, 1926, **60**, 435; 1927, **62**, 17.

[5] Kilbourne, N. J., Dodson, V., and Zeiler, A. H., *Surg. Gynec. and Obst.*, 1932, **54**, 640.

[6] Isaak, L., *Arch. Dermat. and Syphilol.*, 1940, **42**, 86.

[7] Jensen, H., and Jannke, P., *J. Am. Pharm. A. (Scient. Ed.)*, 1944, **33**, 362.

FIGURE 1
DOSE RESPONSE RELATION IN THE VASCULAR OBLITERATION WITH SURFACE ACTIVE COMPOUNDS



between 2 to 7 days were not significant. The experimental points in Fig. 1 are averages of 2 to 3 readings made within 1 week after the injection. Each point represents the average length of the thrombus calculated from the results obtained with usually 10, but not less than 4 mice.

The average length of the thrombus decreased gradually during the latter part of the first week and subsequently. In the low-response range recanalization was frequent. In the high-response range permanent obliteration through organization took place. The extent of obliteration after resorption of the thrombus was measured in a few instances by dipping the tails in warm water, thereby

distending the patent veins, and it was found to be approximately proportional to the obliteration caused by thrombosis.

Materials Used. Extensive tests using 130 mice were made with a commercial sodium ricinoleate solution, pH 8.3, containing 5% of the soap and 2% of benzyl alcohol in water. Dilutions of 0.15 to 2.0% made with sterile distilled water were used.

The sodium morrhuate tested was also a commercial preparation containing 5% of the soap and 2% of benzyl alcohol in water. The solution was slightly turbid and had a pH of 10.1. Dilutions containing 2.0, 1.0, 0.5 and 0.25% of the soap were made with sterile distilled water. Ten mice were injected with each of these dilutions.

Sodium tetradecyl sulfate (sodium 2-methyl-7-ethyl-undecyl sulfate-4) was prepared by purification of the commercial product. It was a colorless, transparent, waxy solid which contained 90% of the detergent; the remaining 10% consisted of phosphate buffer and water. Water-clear solutions of pH 7.5 were made with water or 0.85% aqueous sodium chloride solution. They were sterilized by autoclaving for 20 minutes at 15 lb pressure. This procedure did not cause appreciable hydrolysis of the ester as the solutions remained water clear and their pH value remained the same or decreased only slightly. One hundred and seventy mice were used and the concentration of the solutions injected varied from 0.125 to 2%.

For testing the effectiveness of sodium dodecyl and sodium n-tetradecyl sulfate 0.5% and 0.3% solutions of Duponol were used. The pH of the solutions was 7.1. Ten animals were injected with each of the 2 solutions.

Four of the aerosol type of compounds, the di-2-ethylhexyl (aerosol OT) (I), di-n-octyl (II), diethylpropyl (III), and di-n-amyl (IV) sodium sulfosuccinates were obtained in a highly purified form from the American Cyanamid Company.* Clear, approximately neutral solutions in distilled water were prepared by warming, if necessary, and keeping at 40°C until injected. Ten mice were injected with each of the solutions containing 0.125, 0.25, 0.50 and 1.0% of I; 0.25, 0.50, 1.0 and 2.0% of II; and 1.0 and 2.0% of III and IV.

Results. In Fig. 1 the average length of thrombus is plotted against the logarithm of the concentration. It seems that a correlation exists between the concentration and the response, *i.e.*, the average length of the thrombus. With sodium tetradecyl sulfate and the soap solutions, the response was proportional to the logarithm of the concentration, *i.e.*, dose, up to a response of about 40 mm. The maximal individual response was about 45 mm as this was the length of the vein exposed to the drug. The slope of the rectilinear portion of the dose response curve was about the same for these substances. From the estimated position of the lines, the ratio of potencies, that is, the reciprocal of the ratio of the doses producing equal responses, was estimated to be 1.5 for sodium tetradecyl sulfate over sodium ricinoleate. The preparation of sodium morrhuate used in this study seemed to be considerably weaker in thrombogenic activity than either sodium tetradecyl sulfate or sodium ricinoleate.

The slope of the straight portion of the curve obtained with the 2 isomeric aerosols, I and II, appears to be smaller than the slopes obtained with the soap solutions or sodium tetradecyl sulfate solution, although the difference may not be significant. The branched isomer, I, was found to be a much stronger thrombogenic agent than the normal chain isomer, II. In low concentrations I was more active than sodium tetradecyl sulfate. The dipentyl sodium sulfosuccinates, III and IV, were much less active than the dioctyl compounds. Here the branched chain compound, IV, showed no activity in the concentration used, whereas the normal chain compound, III, showed a slight activity. The mixture of the normal chain sodium dodecyl and tetradecyl sulfate was much less active than the branched chain sodium tetradecyl sulfate.

Toxicity to Tissues. This was tested by injecting aqueous solutions of the compounds in concentrations given in Tables I and II in doses of 0.1 cc subcutaneously and in doses of 0.05 cc intradermally into rabbits. The

* Through the courtesy of Mr. Clyde Sluhan.

TABLE I.
Reactions Produced by the Subcutaneous Injection of Soap Solutions and Sodium Tetradecyl Sulfate. (Figures: diameters in mm; dose: 0.1 cc; reading: 48 hours after injection).

Concentration, %	Edema			Erythema		
	Sor	STS	Morrh	Sor	STS	Morrh
5	15	0	8	15	0	8
2.5	0	0	11	0	0	11
1.25	0	0	8	0	0	8
0.625	0	0	0	0	0	0

Sor: Sodium ricinoleate.
STS: Sodium tetradecyl sulfate.
Morrh: Sodium morrhuate.

TABLE II.
Reactions Produced by the Intradermal Injection of Soap Solutions and Sodium Tetradecyl Sulfate. (Figures: diameters in mm; dose: 0.05 cc; reading: 48 hours after injection).

Concentration %	Necrosis			Hemorrhage			Edema			Erythema		
	Sor	STS	Morrh	Sor	STS	Morrh	Sor	STS	Morrh	Sor	STS	Morrh
5	11	5	8	6	3	7	13	6	10	13	6	15
2.5	10	7	6	0	2	0	14	12	13	14	12	14
1.25	9	3	6	0	0	0	11	3	12	12	8	12
0.625	6	0	6	0	0	0	9	6	6	9	6	11
0.313	4	0	6	0	0	0	6	0	6	6	0	11

Sor: Sodium ricinoleate.
STS: Sodium tetradecyl sulfate.
Morrh: Sodium morrhuate.

results in Table I show that sodium tetradecyl sulfate produced no appreciable irritation upon subcutaneous injection and that it was definitely less irritating than the soap solutions. The intradermal injections did not show significant differences among these compounds although the edema and erythema produced by sodium tetradecyl sulfate appeared to be less than that produced by soaps.

Acute Toxicity. Purified sodium tetradecyl sulfate (Tergitol-4) was found by Smyth *et al.*,[8] to have a toxicity of $LD_{50} = 2$ gm/kg when tested on rats by feeding a 25% aqueous solution by stomach tube. In testing the toxicity of Aerosol OT in mice Lorenz *et al.*,[9] found that 1 mg but not 2.5 mg is tolerated when given intraperitoneally and that 1.25 mg was lethal to 1 out of 25 mice weighing 25-30 g. The oral LD_{50} of Aerosol OT in mice has been reported to be 1.5 g/kg.[10]

The intravenous route of administration was chosen for acute toxicity determinations as this is used for therapy with these substances. The mice were fasted for 24 hours and injections of 0.1 cc of various dilutions were given over a period of 20 seconds. Most deaths occurred within 24 hours and no change of survival rate was found after 1 week. LD_{50} was estimated graphically,[11] to be 90 ± 5 mg/kg for sodium tetradecyl sulfate using 47 mice, 100 ± 30 mg/kg for Aerosol OT using 15 mice, 100 ± 3 mg/kg for sodium ricinoleate using 24 mice, and 150 ± 45 mg/kg for sodium morrhuate using 35 mice. Thus only slight differences were found in the toxicity of these substances. The value obtained for Aerosol OT is in fair agreement with the results of Lorenz *et al.*,[9] ($LD_{50} > 40$ and < 100 mg/kg), in view of the fact that the rate of injection which was not specified by these authors might have been greater than that used in these experiments.

Chronic Toxicity. Smyth *et al.*,[8] reported that sodium tetradecyl sulfate did not pro-

[8] Smyth, H. F., Seaton, J., and Fischer, L., *J. Indust. Hygiene and Toxicol.*, 1941, **23**, 478.

[9] Lorenz, E., Shimkin, M. B., and Stewart, H. L., *J. National Cancer Institute*, 1940, **1**, 353.

[10] Benaglia, A. E., Robinson, E. J., Utley, E., and Cleverdon, M. A., *J. Indust. Hygiene and Toxicol.*, 1943, **25**, 175.

[11] Miller, L. D., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261.

duce appreciable toxicity when approximately 150 mg/kg were taken daily in the drinking water by rats for 30 days. Benaglia *et al.*,[10] gave doses varying from 0.19 to 0.87 g/kg to rats with the food and doses between 0.1 and 0.5 g/kg to rabbits, monkeys, and dogs for 24 hours without finding appreciable toxicity, although some inhibitory effect on the growth rate was discernible.

Repeated intravenous injection with these solutions could not be carried out. Among the practicable routes in mice, intraperitoneal injection most closely approximates the intravenous route. Hence, 0.1 mg sodium tetradecyl sulfate in 0.1 cc was injected into 19 mice first intravenously, then 12 times intraperitoneally in the course of 3 weeks. Four mice died of peritonitis or intercurrent infection during the first week. The average weight of the remaining mice increased from 25.9 g to 31.3 g. Five of the surviving mice showed localized infections (abscess in the spleen, localized peritonitis, and adhesions). These were obviously due to perforation at the time of injection. Sections made of the lungs, liver, kidney, spleen, brain, intestine, stomach, testis, and heart of the remaining 10 mice showed no pathology attributable to the drug.†

Summary. The thrombogenic activity of some soaps and synthetic detergents was compared by injecting their solutions under standardized conditions into the tail vein of mice. Sodium tetradecyl sulfate (2-methyl-7-ethyl-undecyl sulfate-4) and Aerosol OT (di-2-ethylhexyl sodium sulfosuccinate) were found to be more potent agents than the soap solutions generally used to sclerose veins. Alkyl sulfates and sulfonates containing large branched chain hydrophobic residues were found to produce thrombus more readily than their straight chain isomers. Sodium tetradecyl sulfate produced less tissue reaction than sodium ricinoleate or sodium morrhuate. Its toxicity was not significantly greater than that of the soap solutions studied.

† Thanks are due Dr. I. E. Gerber, pathologist, for the preparation and examination of the slides.

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Sulfonamide and Penicillin Resistance of Group A Hemolytic Streptococci.*

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It has frequently been possible to establish satisfactory prophylaxis against Group A hemolytic streptococcus respiratory infection and its complications by the daily administration of a sulfonamide.[1] Such a technic, established by the U. S. Navy in many activities early in December, 1943, was uniformly satisfactory[2] until mid-summer of 1944. At that time strains of hemolytic streptococci that were resistant to the antibacterial action of these agents appeared[3] as the cause of disease among personnel in a station in the northwest and initiated an epidemic in spite of the widespread use of chemoprophylaxis. Later these organisms were transmitted widely to other Navy activities through the nor-

* This study was carried out under the auspices of the Commission on Hemolytic Streptococcal Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

1 U. S. Department of Labor, Children's Bureau Publication No. 308, Washington, 1945, 64-87.

2 NAVMED 284, Bureau of Medicine and Surgery, Navy Department, Washington, D. C., 1944.

3 a. Epidemiology Unit No. 22, *J. A. M. A.*, 1945, **129**, 921; b. Damrosch, D. S., *J. A. M. A.*, 1946, **130**, 124; c. Eckles, L. E., personal communication, Sept. 28, 1945.