thorium dioxide in studying contractions of the spleen in various animals. Tripoli[5] obtained visualization of liver and spleen in dogs, rats, guinea pigs, mice, and rabbits, and Shute and Davis[8] in rabbits and dogs. The latter investigators state that thorium dioxide practically disappears from the liver and spleen in 2 or 3 months and the cells regain almost a normal appearance.

Summary. A method of following the course of liver cirrhosis and splenic enlargement induced by feeding butter yellow to albino rats is described. The procedure consists of intracardiac injection of 0.5 cc of thorium dioxide sol (thorotrast) followed by roentgenograms of the liver and spleen.

7 Haam, V. E., Tripoli, C. J., and Lehman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1056.

8 Shute, E., and Davis, M. E., *Arch. Path.*, 1933, **15**, 27.

14504

Potent Typing Sera Produced by Treatment of Donors with Isolated Blood Group Specific Substances.

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A rise in titer of isoagglutinins has been observed in patients following the erroneous transfusion of incompatible blood. There has been no uniform confirmation of this observation as in some instances the antibody titer did not change. Aubert, Boorman, and Dodd[1] reported an increase in isoagglutinin titer of A patients following the injection of several hundred cc of B plasma and of B patients treated with A plasma.† It would seem to be of interest to find out whether the purified group specific substances also will produce a rise in isoantibody titer when injected into human beings.

The blood group specific substances[2,3,4,5] used in the investigations to be reported were prepared by the Eli Lilly Company. The material consisted of a mixture of AB substance (isolated from the horse stomach) and A substance (isolated from the hog stomach.) Three to 5 mg AB substance and 5 to 10 mg A substance were dissolved in 10 cc of normal saline solution and dispensed in vials after being tested for sterility and potency.

The determination of the titer of the test sera was done in the following way: decreasing amounts of the donor's serum in volume of 0.2 cc were mixed with 0.2 cc of 1 to 2% suspension of washed, freshly-obtained blood cells belonging to different groups. After standing for 15 minutes, the tubes were centrifuged at medium speed for one minute. Reading of the degree of agglutination was done macroscopically after the tubes had been shaken slightly.

In the first series of experiments 3 donors belonging to groups O, A, and B received 10

* Observations made before entrance into active military service while fellow in Clinical Pathology, Buffalo General Hospital, Buffalo, New York.

1 Aubert, E. F., Boorman, K. E., and Dodd, B. E., *J. Path. and Bact.*, 1942, **54**, 89.

† According to personal communication Wiener obtained increases in isoagglutinin titers in at least half of his donors following injection of pooled plasma.

2 Witebsky, E., and Klendshoj, N. C., *J. Exp. Med.*, 1940, **72**, 663.

3 Witebsky, E., Klendshoj, N. C., and Swanson, P., *J. A. M. A.*, 1942, **116**, 2654.

4 Witebsky, E., Klendshoj, N. C., and Swanson, P., *Neutralization of Isoagglutinins Anti-A and Anti-B in O Blood by Means of the Addition of the Isolated Blood Group Specific Substances, Blood Substitutes and Blood Transfusion*, Charles C. Thomas, 1942, 327.

5 Klendshoj, N. C., McNeil, Crichton, Swanson, P., and Witebsky, E., *Arch. Int. Med.*, 1942, **70**, 1.

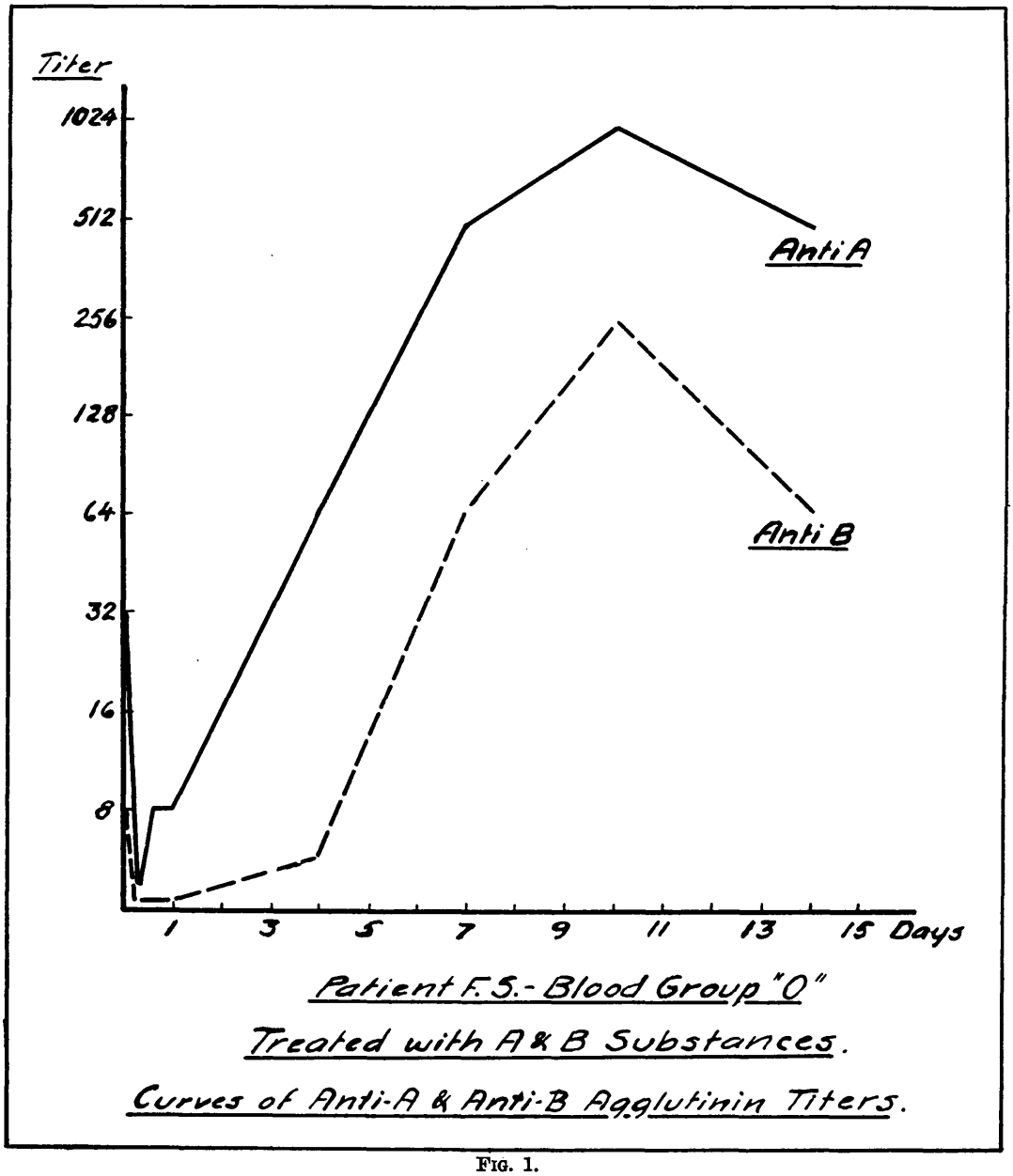

FIG. 1.

cc, or one vial, of the group specific substances, diluted in 100 cc saline solution, by the drip method. Fig. 1 illustrates the change in isoagglutinin titer of the donor belonging to group O following intravenous treatment with A & B substances.

The isoagglutinin anti-A was reduced by the injection of the material from a titer of 32 to 2, the isoagglutinin anti-B from a titer of 8 to 1. After a few days the original titer was restored and the peak was reached 10 to 12 days following injection. A slight decrease in titer occurred during the observation period of two months. Yet after that period the titer was still far above the original.

The isoantibody titer of the second donor (Group B) rose only from 1:16 to 1:64 but the character of agglutination in higher serum

TABLE I.
Agglutination of B Cells by Serum of Patient "G," Group A, Treated with A and B Specific Substances.

Serum dilutions	Before treatment	After* treatment
1. 1:10	++++	++++
2. 1:20	+++	++++
3. 1:40	+	++++
4. 1:80	±	++++
5. 1:160	—	+++
6. 1:320	—	++
7. 1:640	—	++
8. 1:1280	—	++
9. 1:2560	—	+
10. 1:5120	—	±
11. 1:10,240	—	—
12. 0	—	—

* 17 days after termination of treatment.
Symbols: — = no agglutination; ± = very slight agglutination; + = slight agglutination; ++ = moderate agglutination; +++ = strong agglutination; and ++++ = very strong agglutination.

concentrations was much stronger in the serum specimen obtained after treatment as compared with that obtained before treatment.

The third donor belonging to blood group A experienced a rise in isoantibody anti-B from 8 to 256.

In the second series of experiments the treatment was changed and 4 consecutive injections, consisting of 0.1, 1, 3, and 10 cc, were given intravenously. The 0.1 cc used for the first injection was taken up in 1 cc saline solution; the other injections were given with undiluted material.

Table I contains a comparison of serum titrations of a donor belonging to group A before and after intravenous treatment with 4 injections of A and B specific substances. An increase from 80 to a titer of about 5000 was observed in this case.

Subjected to the same treatment one donor did not show any considerable increase in antibody titer. Therefore, it was felt that too much of the A and B substances might have been injected considering the fact that one vial of the A and B substances proved to be potent enough to reduce the antibody titer present in the circulation of donors to the extent shown in Fig. 1. Another donor belonging to blood group O was given only one intravenous injection consisting of 0.1 cc of the stock A and B solution taken up in 1 cc pyrogen-free saline solution. The increase in antibody titer following the intravenous treatment with this small amount of A and B substances is shown in Table II.

The increase in antibody titer following the injection of minute amounts of A and B specific substances (0.03 to 0.1 mg of the purified A and B substances) is considerable. The isoantibody anti-A rose from a titer of

TABLE II.
Serum of Patient "R," Treated with Minute Amounts of A and B Substances.

Dilutions Serum "R," Group "O"	Before treatment		After treatment	
	Agglutination of			
	A cells	B cells	A cells	B cells
1. 1:4	++++	+++	++++	++++
2. 1:8	+++	++	++++	++++
3. 1:16	++	+	++++	++++
4. 1:32	+	±	++++	++++
5. 1:64	±	—	++++	++++
6. 1:128	—	—	++++	++++
7. 1:256	—	—	++++	++++
8. 1:512	—	—	+++	+++
9. 1:1024	—	—	+++	++
10. 1:2048	—	—	+++	+
11. 1:4096	—	—	++	+
12. 1:8192	—	—	+	±
13. 1:16,384	—	—	±	—
14. 1:33,768	—	—	—	—
15. 1:67,536	—	—	—	—
16. 1:134,072	—	—	—	—
17. 1:268,144	—	—	—	—
18. 1:536,288	—	—	—	—
19. 1:1,072,576	—	—	—	—
20. 0	—	—	—	—

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.

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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.