from chick embryos of various ages, and lead to a molecular weight of about 60,000.

Total solids in serum samples from 180 and 250 mm pigs were found to be 17.2 and 17.4 mg/ml respectively after dialysis against H_2O and lyophilization. Nitrogen determinations yielded 12.5% and 13.2% respectively, values which did not change measurably after a portion of the solids were refluxed for 24 hr with alcohol-ether under N, indicating little or no substances soluble in organic solvents. Three small aliquots (1 to 3 mg each) from each sample of lyophilized solid were analyzed for phosphorus but were found to contain only traces.‖

The ratio of carbohydrate to nitrogen in the pig embryo serum, like that in the chick, was high, being 2 to 3 times that in the serum of adult swine. Whether or not all of this is bound to the protein, further investigation will show.

A correlation of these findings with the morphogenesis and organogenesis of the embryos is being made.

Summary. Electrophoresis patterns show rapid changes in the plasma of developing chick and pig embryos. In the ultra-centrifuge only a single component is apparent in the unfractionated plasma. After electrophoretic fractionation of the plasma or sera from older fetuses, traces of other components are revealed.

Diffusion and sedimentation constants of 11-day chick embryo serum would indicate a molecular weight of more than 200,000. The diffusion rate of the plasma from older chick fetuses was greater. Pig fetus plasma had a much larger diffusion constant, indicating a molecular weight of approximately 60,000 for the bulk of the plasma protein from 180 and 250 mm fetuses.

Thirteen-day-old chick serum protein contained 6.0% N and 20 to 30% carbohydrate, whereas 180 mm pig embryo serum protein contained 12.5% N and 15 to 20% carbohydrate.

‖ We are greatly indebted to Miss Helen Fabricant for these analyses.

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Simple Method of Preparing Potent Blood Grouping Sera.*

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The properties A and B are antigenic for human beings whose erythrocytes lack the corresponding agglutinogens, as was demonstrated by observations made following unintentional transfusions of blood of an incompatible group.[1,2] Aubert, Boorman, and Dodd[3] observed a similar increase in isoagglutinin titer in patients transfused with serum prepared from the blood of donors belonging to an incompatible group. Wiener[4] applied this observation as a method of preparing high-titered blood grouping sera, by immunizing professional blood donors with transfusions of dried pooled plasma (Sharp and Dohme) reconstituted with distilled water.† Satisfac-

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1 Wiener, A. S., Oremland, B. H., Hyman, M. A., and Samwick, A. A., *Am. J. Clin. Path.*, 1941, **11**, 102.

2 Wiener, A. S., *J. Immunol.*, 1941, **41**, 181.

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

4 Wiener, A. S., unpublished observations.

† Based on these findings, individuals of group 0 who have previously received plasma transfusions should not be used as universal donors, unless it is proved by careful titration tests that their sera do not contain dangerously high concentrations of isoagglutinins.

tory typing sera were obtained from about half of the donors injected.

A simpler method was developed by Witebsky *et al.*[5] entailing the use of solutions containing a mixture of purified AB substance (isolated from horse stomach) and A substance (isolated from hog stomach). As little as 0.1 cc of the solution containing 3 to 5 mg of AB substance and 5 to 10 mg of A substance in 10 cc of normal saline solution, when injected intravenously induced a marked increase in the isoagglutinin titer. Moreover, a group O individual injected intramuscularly with 1 cc of the solution showed a 100-fold increase in both isoagglutinins anti-A and anti-B. Wiener[6] has confirmed this report of Witebsky *et al.* and found that intramuscular injections gave as satisfactory results as intravenous injections of the purified group substances.

The chief disadvantage of the method of Witebsky *et al.* is that the purified substances are not generally available. There is also a theoretical possibility that improperly prepared material may contain traces of animal protein and give rise to foreign protein reactions in rare hypersensitive individuals. Accordingly, the purpose of the present report is to describe a new, simple and safe method of preparing immune high-titered sera, that can be carried out by any competent worker interested in blood grouping.

The new method of preparing high-titered grouping sera is similar in principle to that of Witebsky *et al.*, but entails the use of solutions of A and B substances derived from human saliva. Blood donors are immunized by intramuscular injections of 1 cc of the partially purified saliva, diluted 1:5 with saline solution, and the donors are bled 2 weeks or longer after the injection, provided their serum shows a satisfactory increase in isoagglutinin titer.

The saliva used by us for the injection was processed as follows. The saliva was autoclaved at 15 lb pressure for 15 min, and the coagulated protein and other insoluble material were then easily removed by centrifuging. The supernatant opalescent fluid was mixed with 4 parts of sterile saline solution, and the diluted saliva was distributed among a number of sterile vials of convenient size. To insure sterility the material was autoclaved a second time in the vials which were then closed with sterile rubber caps. The group substances are apparently not damaged by the processing because the inhibition titer of the "purified" saliva was approximately ⅕ the titer of the original saliva, as expected from the dilution. Incidentally, the use of saliva is advantageous not only because of the ready availability of the material, but also because it excludes the possibility of sensitization to foreign proteins and in addition makes it possible to prepare solutions of the A and B substances separately. Of course one must be certain that the saliva is collected from secretors, but this is no serious problem because 85% of all individuals are secretors. It should be mentioned that if the saliva cannot be processed immediately after collection it should be placed in boiling water for about 10 min in order to destroy the blood group enzymes, and then stored in the refrigerator until processing can be carried out.

Titration experiments showed that the inhibition titer of processed A saliva is about equal to or slightly lower than that of Witebsky's blood group solutions, while processed B saliva is about 4 times as active.

With regard to the antigenic activity of these preparations the following experiment can be cited as an example. Five group O and 3 group A patients were given intramuscular injections of 1 cc of processed group B saliva. After 12 days blood samples were collected and the isoagglutinin titers compared with the original titers of blood taken before the injections. As can be seen from Table I the great majority of the patients responded to the injection with rises in isoagglutinin titers ranging up to 10 times the original value. In some of the group O patients the anti-A titer rose as well as the anti-B titer even though the material injected contained no A substance. This may be explained by postulating the presence in A and B substances of a common property C capable of stimulating the production of specific anti-C isoagglutinins.[7] Or one could assume that properties A and B have certain similarities in chemical structure and

[5] Witebsky, E., Klendshoj, N. C., and McNeil, C., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 167.

[6] Wiener, A. S., unpublished observations.

TABLE I.
Antigenic Effect of Intramuscular Injections of Small Amounts of Autoclaved Group B Saliva.

Patient No.	Blood Group	Initial titer of serum for A_2	Initial titer of serum for B	Titer after saliva injections A_2	Titer after saliva injections B
1	O	6	2	45	60
2	O	6	6	50	90
3	O	2	2	6	4
4	A	0	50	0	490
5	O	1½	3	6	60
6	A	0	7	0	12
7	A	0	75	0	90
8	O	3	25	1½	90

that the cross reactions are due to antibodies of low specificity.[8,9]

It may be mentioned that saliva processed as described in this paper is more satisfactory for inhibiting anti-A and anti-B isoagglutinins in human anti-Rh sera than pooled, boiled saliva as originally recommended.[10]

Comment. According to the classic concept, the group substances owe their specificity to carbohydrate structures, and in the erythrocytes these are supposed to be conjugated with lipids and/or proteins, which confer on the agglutinogens their antigenicity.[7] In the secretions, the group substances are supposed to be present in a soluble form, as haptens free of proteins and lipids and therefore non-antigenic. Actually, our observations cited in this paper refute these ideas. The intramuscular injections of relatively minute quantities of saliva have been shown to stimulate substantial rises in isoagglutinin titer. On the other hand, injections of comparable or larger amounts of erythrocytes by the intramuscular route appear to have little or no stimulating effect. In fact, Wiener[11] has injected substantial amounts of red cell stromata intramuscularly without producing any appreciable change in isoagglutinin titer. Obviously, our concepts concerning the nature of the group substances in erythrocytes and secretions will have to be revised.

Witebsky's investigations have demonstrated the feasibility of preparing large quantities of purified A and B group substances from animal sources, and such material will undoubtedly soon become generally available. The A and B substances from human saliva will hardly replace the excellent product from animal sources, but may serve as a temporary stop-gap in emergencies wherever the commercially prepared A and B substances are difficult or impossible to obtain.

Conclusions. The intramuscular injection of small amounts of autoclaved saliva (0.2 cc) from secretors of groups A and B often stimulates a considerable increase in the isoagglutinin titer. This method simplifies the large-scale production of potent blood grouping sera.

[7] Wiener, A. S., *Blood Groups and Transfusions*, 3rd ed., p. 293, C. C. Thomas, Springfield, Ill., 1943.

[8] Wiener, A. S., and Karowe, H. E., *J. Immunol.*, 1944, **49**, 51.

[9] Boyd, W. C., and Warshaver, E. R., *J. Immunol.*, 1945, **50**, 101.

[10] Wiener, A. S., and Forer, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 215.

[11] Belkin, R. B., and Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 214.