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Effect of a Modified Kaolin Treatment on Serum Immunoglobulins

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After kaolin treatment of fetal rabbit serum, 7*S* antibody titers were reduced more than 19*S* titers. This reduction was less when the kaolin treatment was performed at *p*H 9.0 than when it was performed at *p*H 7.3. A modification of the kaolin treatment of sera for use in the hemagglutination-inhibiting antibody titration, in which the hemagglutination reaction is performed at a neutral *p*H, is recommended. The advantage of the modified method is that adsorption of immunoglobulins to kaolin is minimized when serum is treated at a lower dilution with *p*H 9.0 kaolin, followed by reduction of the *p*H of the supernatant fluid to neutrality with a "serum adjusting diluent." When the serum was diluted with physiological saline before kaolin treatment, a great decrease in serum immunoglobulin concentrations occurred. This decrease was found to be less in the modified kaolin treatment than in the conventional *p*H 7.3 kaolin treatment.

It has been generally believed (5, 8) that kaolin treatment of serum for the removal of nonspecific hamagglutinin inhibitors reduces hemagglutination-inhibiting (HI) antibody titers, especially those of immunoglobulin M (IgM) antibody, since the report of Mann et al. (7). However, these authors did not state that IgM antibodies were more readily adsorbable to kaolin than were the other classes of immunoglobulins.

During a study of the infection of pregnant rabbits with rubella virus, it was found that 19*S* as well as 7*S* rubella HI antibodies were transmitted from the mother to the fetal serum and gastric juice and to the amniotic fluid [J. Immunol., in press; see also Brambell and others (1, 6) concerning transmission of rabbit IgM and IgG]. During this study, it was observed that kaolin treatment greatly reduced the antibody titers, especially that of 7*S* antibody, of the fetal rabbit serum and gastric juice, but not of the maternal rabbit serum.

In this communication, the recommendation is made that great care should be taken when kaolin treatment is used with specimens having a different protein composition than that of ordinary serum. A modification of kaolin treatment for HI tests where the hemagglutination reaction is performed at neutral *p*H is also suggested.

MATERIALS AND METHODS

Antibody production in pregnant rabbits and harvesting of body fluids from fetuses. Pregnant Japanese white rabbits, NIBS strain, supplied by the Nippon Biological Science Institute, Tachikawa, Tokyo, Japan, were intraveneously infected with rubella virus, Brown strain, $10^{3.7}$ $TCIntD_{50}$ (50% tissue culture interfering dose), on day 8 of gestation. For the production of reference hemolysins, rabbits were, in addition, given repeated injections of 5 ml of a 10% sheep erythrocyte suspension on days 9, 13, 17, and 21 of gestation. On day 28, the animals were killed. The maternal and fetal serum, the fetal gastric juice, and the amniotic fluid were collected.

Kaolin treatment. One part of the undiluted specimen was treated with three parts of a 25% kaolin suspension in phosphate-buffered saline without Ca^{++} and Mg^{++} (PBS, *p*H 7.3; reference 4) or in borate-buffered saline (*p*H 9.0; reference 3). The supernatant fluid was regarded as a 1:4 dilution, according to convention, though it was not a true 1:4 dilution because kaolin is insoluble. The control consisted of serum diluted fourfold with physiological saline. Both samples were subjected to centrifugal analysis.

For comparison, human sera were tested with the following two methods of kaolin treatment; both the sequence of treatment and the *p*H are different in the two methods.

Modified method. A 0.2-ml amount of serum was pipetted into a small test tube, and 0.8 ml of a 25% kaolin suspension in borate-buffered saline (*p*H 9.0)

was added. The mixture was shaken at room temperature for 20 min, followed by centrifugation at 2,000 rev/min for 20 min. A 0.6-ml amount of a "serum adjusting diluent" (0.05 M sodium phosphate, *p*H 6.3, 0.15 M NaCl), with which the *p*H of the supernatant fluid can be reduced to neutrality, was added. The supernatant fluid was decanted to another tube, and natural agglutinins were absorbed with 0.05 ml of a 50% erythrocyte suspension. This supernatant fluid is regarded as a 1:8 dilution of the original serum.

Conventional method. A 0.2-ml amount of serum was pipetted into a tube; 0.6 ml of PBS (*p*H 7.3) was added, followed by the addition of 0.8 ml of a 25% kaolin suspension in PBS. The remainder of the procedure was the same as the modified method except that the addition of "serum adjusting diluent" was omitted.

Sucrose gradient centrifugation. A 0.5-ml amount of specimen, diluted 1:4, was layered on a 4-ml 12.5 to 37% (w/v) sucrose-PBS gradient, and was centrifuged at 35,000 rev/min for 16 hr at 5 C in a Spinco SW39 rotor. After centrifugation, five-drop fractions were collected from the bottom of the tube.

Rubella HI antibody titration. The original method of Stewart et al. (9) was slightly modified. The diluent used in this study was Veronal-buffered saline (NaCl, 8.5 g; sodium barbital, 0.375 g; barbital, 0.575 g, distilled water, 1,000 ml; *p*H 7.3) to which was added $CaCl_2$ (0.02%), $MgCl_2 \cdot 6H_2O$ (0.02%), bovine serum albumin (0.1%), and gelatin (0.01%). This diluent was also used for the titration of hemolysins. All tests were performed by use of the microtiter method with U-type microplates (S-MRC-96, Linbro Chemical Co., New Haven, Conn.).

Hemolysin titration. A 50-μliter amount of complement (2 hemolytic units) and 25 μliters of a 2% sheep erythrocyte suspension were added to a 25-μliter volume of the specimens previously diluted in a twofold series in the microplates. The microplates were then sealed with tape and incubated in a water bath at 37 C for 1 hr with occasional shaking. After incubation, the plates were centrifuged in special centrifuge carriers (Cooke Engineering Co., Alexandria, Va.). The hemolysin titer was read as the dilution giving 50% hemolysis.

Determination of immunoglobulin levels. Concentrations of human immunoglobulins (IgG, IgM, and IgA) were determined by a single radial immunodiffusion method with the use of Immuno-Plates

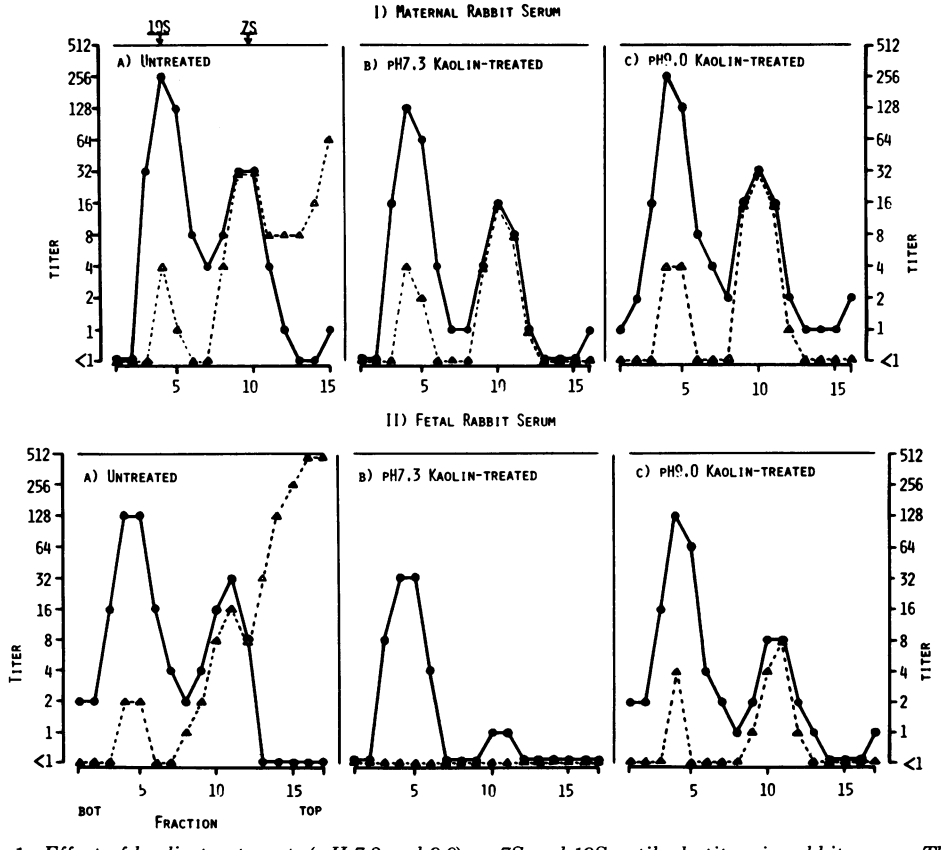

FIG. 1. *Effect of kaolin treatments (pH 7.3 and 9.0) on 7S and 19S antibody titers in rabbit serum. The 7S antibody titers in the fetal serum were greatly reduced when it was treated with pH 7.3 kaolin, but the reduction was less with pH 9.0 kaolin. However, the titers in maternal serum were not diminished as much with either treatment. Symbols: △, rubella HI titer; ●, hemolysin titer.*

(Hyland Laboratories, Los Angeles, Calif.) for low-level immunoglobulins.

RESULTS

Effect of kaolin suspension pH on antibodies in fetal rabbit serum. Figure 1 shows the sedimentation patterns of kaolin-treated specimens of maternal and fetal rabbit serum. The 7*S* antibody titers of the fetal serum were greatly reduced when the serum was treated with kaolin at *p*H 7.3, but were not reduced so much when treatment was at *p*H 9.0. The same patterns were also obtained in the case of treatment of fetal gastric juice. When amniotic fluid was treated with kaolin, both 7*S* and 19*S* antibody titers were reduced to nearly undetectable levels at either *p*H. However, the titers in the maternal serum were not diminished so much at either *p*H (Fig. 1). Both treatments completely removed the nonspecific hemagglutinin inhibitors which remained in the top fractions after centrifugation of the untreated serum.

Suggested modification of kaolin treatment. From the above data, it might be said that kaolin treatment is better done at *p*H 9.0 than at neutrality, and a modified method of kaolin treatment for the HI tests in which the hemagglutination reaction is performed at a neutral *p*H is suggested.

To determine the validity of this modification, experiments were performed on human cord and adult sera.

First, rubella HI antibody titers in human cord sera were determined after kaolin treatment by the modified and conventional methods given in Materials and Methods. It is well known that human cord sera do not usually contain 19*S* antibodies. Accordingly, experiments were designed to determine whether 7*S* antibody titers in cord sera were affected by kaolin treatment, as was found for 7*S* antibody titers in fetal rabbit sera. Among 20 specimens examined, no difference in titers after treatment by the two methods was found.

Second, rubella HI antibody titers and immunoglobulin levels of adult human sera were examined after the treatments. In this experiment, six specimens of adult human sera which had high-titered HI antibodies were pooled, and twofold serial dilutions were made with physiological saline before the treatments. When the serum was not diluted before the treatments, there was no difference in the HI titers between the two methods (Table 1). However, the more highly the serum was diluted, the greater the adsorption of immunoglobulins to kaolin was. The reduction

TABLE 1. *Effect of serum dilution on the adsorbability of immunoglobulins to kaolin*

Serum[a] dilution	Treatment[b]	Rubella HI titer			Immunoglobulin concn (mg/ml)		
		Observed (A)	Expected (B)[c]	A/B	IgG	IgM	IgA
Undiluted	NT	—	—	—	130 (0)[d]	10 (0)	24 (0)
	Mod	512	—	—	86 (34)	5.6 (44)	20 (17)
	Con	512	—	—	80 (39)	5.0 (50)	18 (25)
1:2	NT	—	—	—	66 (0)	5.0 (0)	10 (0)
	Mod	256	256	1/1	48 (27)	2.0 (60)	8.0 (20)
	Con	128	256	1/2	36 (45)	<2.0 (>60)	5.7 (43)
1:4	NT	—	—	—	36 (0)	3.0	5.7 (0)
	Mod	64	128	1/2	11 (69)	<2.0	4.4 (23)
	Con	32	128	1/4	5.0 (86)	<2.0	3.7 (35)
1:8	NT	—	—	—	14 (0)	—	3.7
	Mod	8	64	1/8	2.0 (86)	—	<3.0
	Con	<8	64	<1/8	<2.0 (>86)	—	<3.0
1:16	NT	—	—	—	7.0	—	—
	Mod	<8	32	—	<2.0	—	—
	Con	<8	32	—	<2.0	—	—

[a] An adult human serum pool was diluted with physiological saline prior to the kaolin treatments.

[b] NT: no treatment; the specimen was diluted eightfold with saline. Mod, Con: modified or conventional method as described in Materials and Methods.

[c] Expected value if there were no adsorption of antibodies in higher dilutions.

[d] Figures in parentheses are the per cent reduction of immunoglobulin levels for each treatment when the NT levels were regarded as 0%.

of the HI antibody titers and the immunoglobulin levels was less when the serum was treated at *p*H 9.0 in the modified method than at *p*H 7.3 in the conventional way.

DISCUSSION

Figure 1 shows that, contrary to most reports (5, 8), IgM antibody is not necessarily more adsorbed to kaolin than IgG antibody. Figure 1 also shows that the IgM and IgG antibody titers in the untreated fetal rabbit serum were almost the same as those in the untreated maternal serum. This probably means that the immunoglobulin content in the fetal serum is almost the same as in the maternal serum. However, the immunoglobulin levels in the fetal serum were greatly reduced after kaolin treatment, which was not the case with the maternal serum. This is probably because the amounts of other proteins in the fetal serum, which protect the immunoglobulins from their adsorption to kaolin, are small, if any. Cabasso et al. (2) reported that kaolin treatment removed considerable amounts of gamma globulins from pure gamma globulin preparations containing protein at levels equivalent to that found in human sera. However, the addition of bovine albumin to some extent prevented the adsorption of the antibodies to the kaolin. Consequently, care should be taken when kaolin treatment is used with body fluids which have protein compositions different from ordinary serum, such as cerebrospinal, ascitic, or amniotic fluid.

Jo et al. (19th Annual Meeting of the Society for Japanese Virologists, Tokyo, October, 1971) examined rubella HI antibody titers of 224 pairs of human maternal and cord sera using the PBS-kaolin treatment. They found that the average HI titer of the maternal sera ($=2^{4.25}$) was higher than that of the cord sera ($=2^{3.64}$). However, they found no difference in titers between the paired sera without kaolin treatment when they examined fluorescent-antibody (FA) titers by use of the indirect FA method in which rubella virus-infected RK-13 cells were used as an antigen. From the results of this study, it is suspected that the lower HI antibody titers in the cord sera might have been caused by the higher adsorption to kaolin of antibodies in those sera.

Schmidt and Lennette (8) reported that kaolin treatment of human sera at *p*H 9.0 sometimes gave higher rubella HI antibody titers than when treatment was at *p*H 7.3. Although in this study with undiluted human cord and adult sera there was no difference in the HI titers between the two treatments, it might be said that the modified method has an advantage in some instances, such as in treating the sera of hypoproteinemic or dysproteinemic patients. The modified method could be used not only in the rubella HI antibody titration but also in case of other viruses, such as adenovirus, enterovirus, reovirus, and measles virus, with which the hemagglutination reaction is performed at a neutral *p*H, as far as their nonspecific hemagglutinin inhibitors are known removable by kaolin treatment at *p*H 9.0.

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