Kelcoloid fed cats indicated that the lesions which were found were of no significance insofar as any specific disease process was concerned. Kelcoloid appeared to be a wholesome food.

Discussion. The algins are partly digestible, but it is not known whether the animal directly or bacteria in the intestinal tract utilize these products. This is of no great nutritional significance since the products are used in only very small quantities in foods.

The difficulties encountered with diets containing high levels of algin may be attributed to the high water absorbing and bulking capacities of this high viscosity product. At the levels fed, water would produce a sticky, heavy paste or semi-gel causing difficulty in chewing and swallowing or a reduction or normal nutrient intake. This kind of physical effect may be expected with any product of this type. Nilson and Schaller(2) reported similar conditions when Irish moss was fed to rats.

In commercial usage the products are, of course, fully hydrated and used at levels of 0.1 to 0.5%. Thus the lowest level fed in these experiments, namely 5%, represents 10 to 50 times the amount that would be consumed in the daily diet of humans if everything eaten contained algin.

Conclusions. The data indicate that these algin products are free from any deleterious substances which adversely affect health when fed at levels which are at least 10 times higher than when used in foods or pharmaceutical products. The difficulties experienced with feeding the higher levels seem to be due, at least in greater part, to the physical texture imparted to the diets fed. This should not be a problem in the commercial use of the product. The data indicate that the three algin products which were tested are wholesome.

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Reversible Agglutination of Trypsin Treated Erythrocytes by Normal Human Sera.* (18581)

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Human erythrocytes, when treated in various ways, are susceptible to agglutination by apparently normal, compatible human sera. This effect is entirely independent of the usual blood groups and their agglutinins. Thomsen (1) first demonstrated that red cells exposed to certain bacteria can be agglutinated by most human sera. Friedenreich(2) extended these observations by showing that an enzyme derived from the bacteria was responsible for the change in the properties of the red cell. Since then it has been shown that exposure of red cells to Cholera Vibrio filtrates(3), viruses of the mumps-influenza group(3), and periodate ions(4) renders these cells panagglutinable by human sera. That the agglutinins in human sera responsible for these effects are varied and distinct has been shown by Stewart(5) and Moskowitz and Treffers (6). Brief mention is made by Wheeler *et al.*

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1. Thomsen, O. Z., *Immun. Forsch.*, 1927, v52, 85.

2. Friedenreich, V., *Compte Rendu. Soc. Biol.*, Paris, 1928, v98, 894.

3. Burnet, F. M., and Anderson, S. G., *Aust. J. Exp. Biol. and M. Sci.*, 1947, v25, 213.

4. Burnet, F. M., In Briody, B. A., *J. Immunol.*, 1948, v59, 115.

(7) of the fact that erythrocytes exposed to the action of trypsin may be agglutinated by normal sera. They recognized that such an effect may not be entirely non-specific but may be related to the agglutination described by Thomsen. During the course of investigating the properties of trypsin-modified red cells, we have observed that these cells are capable of agglutination upon exposure to most human sera. No question of the usual compatibilities existed since this effect was noted even when the trypsinized cells were exposed to their own serum.‡ Agglutination titers of this sort ranged from 1 to 32 units. The character of the agglutinates was distinctive. They formed solid aggregates in undiluted or weakly diluted sera, and finer clumps with higher dilutions. They appeared only if the serum-cell mixtures were spun soon after contact. Incubation of serum-cell mixtures at room temperature for one hour did not produce spontaneous agglutination, nor did centrifugation then reveal agglutination. Observation of the agglutinates formed after immediate centrifugation revealed that they broke up spontaneously and became redispersed on continued contact with the serum mixtures. It appeared then that the agglutination observed was evanescent. The properties of the serum agglutinin as well as of the agglutination reversal effect were then studied.

Methods and materials. Blood samples were drawn from the antecubital vein and allowed to clot at room temperature. The serum was separated from the clot which was used to make the cell suspensions. Both cells and serum were used within 3 hours. In order to insure perfect compatibility, cells from an individual were used against his own serum.

Technic of trypsin modification of the erythrocyte. A modification of the method of Wheeler *et al.*(7) was used. Instead of whole blood, twice washed erythrocytes were used to make up 2% cell suspensions.§ Following exposure to 0.1% crude trypsin (Difco 1:250, product No. 8152) for 10 minutes at 37°C, the erythrocytes were twice washed again with .85% sodium chloride and resuspended to their original volume.

Agglutination tests. Agglutination tests were performed by adding two drops of the trypsin modified cell suspension to two drops of serum or saline dilutions of serum. Following one to 2 minutes incubation at room temperature, the tubes were centrifuged at 1500 R.P.M. for one minute and read for agglutination. The tubes were then vigorously tapped to redisperse the agglutinates as completely as possible. The mixtures were then incubated at room temperature for 5 minutes, recentrifuged, reread, and redispersed. This was repeated at 5 minute intervals for the first 30 minutes, and at 10 minute intervals for the last 30 minutes. As controls, diluted serum and 2% trypsin modified erythrocytes were held apart at room temperature and mixed at the end of an hour (Control A). In Control B, trypsin modified erythrocytes in contact with the serum-saline mixtures but undisturbed were also held for a similar period. Both sets of controls were then centrifuged and read for agglutination. In order to ascertain whether loss of agglutination was due to loss of agglutinin or to an alteration in the trypsin modified erythrocytes, these cells were kept in contact with serum dilutions until agglutination could no longer be demonstrated. The tubes were then centrifuged for 5 minutes and the serum dilutions carefully pipetted off into another set of tubes. To each residual button of red blood cells, 2 drops of fresh undiluted serum were added. To the removed serum dilutions, 2 drops of fresh trypsin modified cells were added. Both sets were shaken, incubated at room temperature for one minute and then centrifuged and read for agglutination. All agglutinations were read with the aid of a hand lens by one observer to insure uniformity.

5. Stewart, F. S., *J. Path. Bact.*, 1949, v61, 456.

6. Moskowitz, M. and Treffers, H. P., *Science*, 1950, v111, 717.

7. Wheeler, W. E., Luhby, A. L., and Scholl, M. L. L., *J. Immunol.*, 1950, v65, 39.

‡ No patient was included in this study in whom complete or incomplete iso- or auto-antibodies could be demonstrated by the usual technics.

§ The use of washed cells rather than whole blood resulted in a greater trypsin action, since the antitryptic activity of plasma is not encountered.

TABLE I. Trypsin Modified Erythrocytes and Serum Dilutions Respun at Stated Intervals.

Time after initial contact, min.	Undiluted	Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64
1	+++	++	++	±	—	—	—
5	+++	++	++	±	—	—	—
10	+++	++	++	±	—	—	—
15	—	++	++	±	—	—	—
20	—	±	++	±	—	—	—
25	—	±	+	±	—	—	—
30	—	—	+	±	—	—	—
40	—	—	±	±	—	—	—
50	—	—	—	±	—	—	—
60	—	—	—	—	—	—	—
Control A	+++	++	++	+	—	—	—
Control B	—	—	—	—	—	—	—

TABLE II. Determination of Serum Activity and Reactivity of Trypsin Modified Erythrocytes After 1 Hour Incubation.

	Undiluted	Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64
Residual button of serum-exposed* trypsin modified erythrocytes + 2 drops fresh undiluted serum	—	—	—	—	+	+++	+++
Removed serum-saline mixtures + 2 drops fresh trypsin modified erythrocytes	+++	+++	+	+	—	—	—

* Serum dilutions to which erythrocytes were exposed 1 hr are indicated by the heading.

Results. The results of the first procedure (Table I) show that with the passage of time, agglutination disappears first in the tubes with the highest serum content and then eventually from all the tubes. Admixture of the trypsin modified cells and serum dilutions *held apart* for a similar period (Control A) shows no such loss of agglutination. However, trypsin modified cells and serum dilutions *held together* but undisturbed (Control B) show a loss of agglutinability. It was evident that contact between cells and serum was necessary for loss of agglutination. Centrifugation was not an important factor.

The results of the experiment designed to demonstrate whether loss of agglutination was due to loss of the serum agglutinin or to an alteration in the trypsin modified erythrocytes are expressed in Table II. It may be seen that the agglutinin had not been lost in the serum dilutions. Rather the trypsin modified erythrocytes exposed to the first four serum dilutions and to a lesser extent to the fifth serum dilution had undergone an alteration making them unresponsive to the further action of the serum agglutinin. A critical amount of serum is necessary for this effect to occur since cells exposed to serum dilutions above 1:32¶ did not show this alteration at the end of one hour. It cannot be stated whether the serum contains a factor that directly brings about this altered reactivity of the treated erythrocytes or whether it accelerates a change on the red cell surface which occurs spontaneously at a slow rate.

Further observations on this agglutination reversal effect revealed that elevation of the temperature under which the effect was studied

¶ That dilution of normal sera to 1:32 often resulted in a loss of agglutination activity as well as loss of the agglutination reversal effect was coincidental. Sera from certain ill individuals have demonstrated an agglutination titer against trypsin modified cells as high as 1:256 although the agglutination reversal effect was seen only up to serum dilutions of 1:32.

hastened the redispersal of the agglutinates while lowered temperatures inhibited this action. A study on the effect of various physical and chemical agents on the agglutinin and the agglutination reversal effect demonstrated certain differences between the two. The agglutinin was non-dialysable, destroyed by heating to 65°C for one hour, and only weakly active at extremes of pH. In contrast, the agglutination reversal effect was lost through dialysis, was thermostabile, and was still active in markedly alkaline serum. The agglutinin could be absorbed from serum with trypsin modified cells and then eluted into saline. It was not possible to do this with respect to the agglutination reversal effect.

Studies on the trypsin modified cells before and after exposure to serum demonstrated that they lost only their reactivity to the panagglutinin found in most sera. Their ability to react with other hemagglutinins, either complete, or incomplete, was unimpaired. Thus they are perfectly adequate as test cells for the detection of hyperimmune antibodies(8).

Discussion. The presence of agglutinins in most human sera, active against modified erythrocytes, may at times be of pathologic significance. Burnet and Anderson have suggested that *in vivo* modification of red cells by exposure to viruses or bacteria may induce a hemolytic episode. Similarly it is possible that proteolytic enzymes released from necrotic, neoplastic, or infected tissue may modify the contiguous erythrocytes in such a fashion as to render them susceptible to the action of agglutinins present normally in all sera. The modification of these cells in itself may in turn stimulate the formation of more agglutinins although it is not certain that such agglutinating bodies develop initially in response to an antigenic stimulus such as virus altered erythrocytes(9).

Practically, the ability of most normal sera to agglutinate trypsin modified red cells offers no deterrent to their use in searching for abnormal antibodies of the so-called blocking or incomplete type. We have successfully used these cells in the investigation of sera from erythroblastotic mothers and cases of acquired hemolytic anemia. One must, however, observe the precaution of incubating the cell-serum mixtures for at least an hour and preferably at 37°C in order to obviate the effect described above.

Summary. An agglutinin is described which exists in most normal sera and which is capable of agglutinating trypsin modified erythrocytes. By virtue of such action, it resembles the agglutinins active against erythrocytes altered by bacterial filtrates, viruses, and periodate ions. Undiluted serum or serum in weak dilution is able to reverse the activity of this agglutinin. Such a reversal effect is mediated through an altered reactivity of the trypsin treated erythrocytes with respect to the agglutinin described above. No modification of the reactivity of the cells with respect to other agglutinins has been noted. The effect of various physical and chemical agents on the activity of the agglutinin and the agglutination reversal effect is described and the patho-physiological role of the agglutinin is discussed.

8. Morton, J. A., and Pickles, M. M., *Nature*, 1947, v159, 779.

9. Lind, P., and McArthur, N., *Aust. J. Biol.*, 1947, v25, 247.

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