

rather sparse literature dealing with CO in plants. From his own experiments he has concluded that light, chlorophyll, and O<sub>2</sub> together are responsible for the concurrent production of CO and aldehydes via a photodegradative process. He further associates injured tissues particularly with CO formation.

Our experiments show that neither light nor chlorophyll is necessary for CO formation, and that, although some oxygen may be required, high levels do not favor its production. Carbon monoxide is formed both by seeds and intact, growing plants. Such results suggest the operation of novel fermentations in higher plants and constitute an extension rather than a contradiction of Wilks's observations.

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### Equilibration of Isoagglutinins of Human Group O Serum

**Abstract.** Cross-reactive antibodies are shown to be responsible for the difference in final levels of agglutination reached after dissociation of a centrifuged group O serum-erythrocyte mixture as compared with duplicate mixtures allowed to aggregate freely. This difference is seen only with certain group O sera, it is independent of complement, and it may be eliminated by absorption of the serum with A or B erythrocytes. The phenomenon is most likely the result of steric inhibition of smaller, cross-reactive antibodies in the aggregative system.

When equal volumes of an antiserum dilution and a suspension of red blood cells are mixed and allowed to aggregate freely (association), the percentage of agglutinated cells eventually reaches a stable maximum level. A final maximum response is also achieved when a duplicate reaction mixture is first centrifuged and the sedimented cells are dispersed during continuous agitation

(dissociation). The system is considered to have attained a true and stable equilibrium when the percentage of agglutinated cells is the same by the two methods (1).

Isoagglutinins from unstimulated group A and group B persons characteristically reach a true equilibrium when they are allowed to react with group B and group A cells respectively. The equilibration curves for the isoantibodies of many unstimulated group O sera, however, show a consistent lack of true equilibrium even though stable levels of agglutination are produced by both the associative and dissociative methods. Quantitative hemagglutination assays can be successfully accomplished only when the system has reached a stable equilibrium. It was considered worthwhile to determine which, if either, of the two levels obtained with group O serum represents a true measure of agglutinating antibody. It appears that the dissociative method is preferable when a measure of total antibody is required.

Sera were obtained from male and nulliparous female donors with no history of recent immunization. Each serum was tested for cross-reactive antibody by the mixed-cell agglutination method of Jones and Silver (2). Equilibrium studies were performed by mixing 0.5 ml of a dilution of antiserum that would produce about 80 percent agglutination with a suspension of red blood cells in 0.25 percent saline. The resulting mixture contained 1.0 to 1.3  $\times 10^4$  cells per cubic millimeter. Unagglutinated cell controls were prepared by substituting saline for the serum dilution.

The serum-cell mixtures for the associative and dissociative assays were prepared identically. In the dissociative assay, the tubes were centrifuged for 1 minute at 200 grav and were then placed along the circumference of a Dacie agitator wheel (3) 11 inches in diameter and rotating at a constant speed of 10 rev/min. The same procedure was followed in the associative assay except that centrifugation was omitted.

Quadruplicate hemocytometer counts of the number of free cells were made periodically from duplicate tubes obtained from each of the two assays. Tubes were sampled once and discarded.

The results with one serum are shown in Fig. 1. This serum was representative of 11 tested and was selected for illustration because the titers with A and B

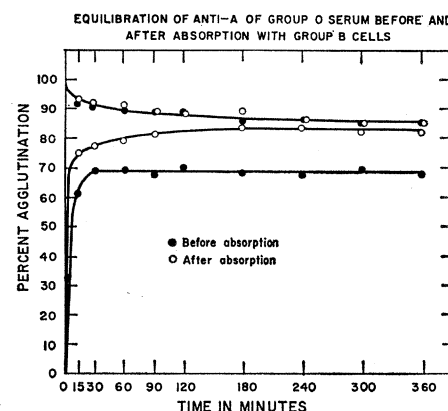


Fig. 1. Equilibration of anti-A of group O serum before and after absorption with group B cells. Note also that dissociation (upper curve) proceeds at the same rate before and after absorption while association is more rapid after absorption (middle curve) than before (lower curve).

cells were equal, avidity times were the same, and cross-reactivity was detectable. Since the results with A and B cells were similar, only those obtained with A cells are given for simplicity. The untreated serum had an anti-A titer of 32 units by the customary serial dilution titration. The slide avidity time at this dilution was 5 seconds. An antiserum concentration of 0.0312 ml/ml was employed in the two assays and gave values of 69 percent agglutination in the associative assay and 84.7 percent in the dissociative assay.

After seven absorptions with one-half volume of packed, washed group B cells, the anti-B activity was completely removed and the anti-A titer was reduced to 8 units. The avidity time was increased to 12 seconds after absorption. Sera absorbed with group O cells gave the same results as unabsorbed sera.

Equilibrium studies with the absorbed serum (0.125 ml/ml) showed that the system could now attain an essentially true equilibrium. As shown in Fig. 1, endpoint values for the assays were within 2 percent of identity. An important feature to be noted is that dissociation follows the same course with absorbed and unabsorbed sera and results in identical curves.

In order to rule out any effects of complement, fresh sera were compared with sera in which complement activity had been removed. Two methods were used for de complementation: (i) heat treatment at 63°C for 10 minutes and (ii) absorption with a washed immune precipitate prepared from the reaction of crystalline bovine serum albumin (BSA) with rabbit anti-BSA (4). In each

case the original difference between the associative and dissociative levels remained unchanged.

The possibility that different thermal optima exist for each of the two types of antibody influencing the assays was eliminated when it was found that assays at 4°, 25°, and 37°C all yielded similar results.

The reasons underlying less complete associative agglutination have not yet been recognized with certainty. Clearly they are related to the properties of the cross-reactive antibodies of group O serum, since in those untreated group O sera lacking a cross-reactive component, the levels of agglutination were identical by both methods.

The elegant work of Jones and Kaneb (5) has shown that the site at which the cross-reactive antibody reacts on the erythrocyte is the same as or extremely close to that reactive with monospecific  $\alpha$  and  $\beta$  molecules. Kochwa *et al.* (6) have shown that the cross-reactive antibodies of group O serum sediment in the 7S fraction while the monospecific agglutinins are confined principally to the 19S fraction. Thorbecke and Franklin (7) demonstrated differences in the specificity of 7S and 19S rabbit antibodies, and Taliaferro and Taliaferro (8) noted avidity differences in rabbit anti-sheep hemolysins of different ultracentrifugal fractions. Winstanley *et al.* (9) have also reported that the hemolysins of group O sera differ in their specificity from the agglutinins.

One might assume then that the cross-reactive antibody, a 7S molecule, has a lesser affinity for the antigen than the 19S component and that centrifugation enhances this affinity. There is no apparent reason, however, why centrifugation should affect affinity and, furthermore, the two levels of agglutination would be expected to equalize in time.

A more likely explanation, based on the differences in the molecular sizes of the two components, is that centrifugation allows the "shorter" cross-reactive antibodies to form cross linkages between the cells by bringing the cells into more intimate contact. The less complete associative agglutination would thus be a function of the molecular size of the antibody, perhaps influenced by the number and positioning of reactive sites on the erythrocyte surface and by electrostatic factors (10).

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#### Possible Continental Continuation of the Mendocino Fracture Zone

**Abstract.** The Mendocino fracture zone may continue through or below North America near 40°N latitude. This is suggested by instances of left-lateral movement, location of basin deeps, sedimentation patterns, interruption of a major magnetic anomaly, and inflection of major structural trends. A major segmentation of middle North America results.

Although none of the great fractures in the northeastern Pacific Ocean have been traced definitely into the North American continent, Menard (1) noted that many of the north-south trending structures along the west coast seem to be confined to continental blocks bounded by eastward projections of several of the fractures.

The Mendocino fracture, one of the great faults of the eastern Pacific, trends almost due west from California for 1200 miles along the 40th parallel, then veers toward the southwest (1). It shows a left-lateral movement (north side westward relative to the south side) recently estimated to be about 750 miles (2). If we arbitrarily continue the trend of the Mendocino fracture eastward through North America near the 40th parallel, there appears to be a series of coincidences at this latitude which suggests that the fracture continues through or below the continent as a zone of left-lateral movement and of weakness.

In 1955, Kelly (3) suggested that the Uinta Range just north of the 40th parallel had moved westward relative to the Colorado Plateau to the south.

Lovering and Goddard (4) interpreted the structure of the Front Range in Colorado to indicate that the area north of a transition zone near Colorado Springs had moved westward relative to the area on the south. However, on the basis of a stress-strain analysis, there is reason to believe that the transition zone is nearer the latitude of Boulder very near the 40th parallel. Norton (5) felt there had also been similar left-lateral movement near the 40th parallel in southeastern Pennsylvania.

With one exception, all of the major basins which occur in the central United States between Nevada and the Appalachians have their deepest parts near the projected trend (as can be seen on the Tectonic Map of the United States, 1961). Furthermore, several of the deeps occur near the intersection of the east-west zone and a major north-south trending uplift. The Currie Basin, in which there are preserved the youngest preorogenic Mesozoic rocks reported in northeastern Nevada (6), is just north of 40°N lat and has been interpreted by Robert Nelson (unpublished manuscript) as "... a broad synclinal downwarp, possibly analogous to the present Allegheny Basin." The deepest part of the Uinta Basin in northeastern Utah is near the intersection of the east-west zone and the north-south trending Central Rockies. The Piceance Basin's deep is near the junction of the projected fracture zone and the White River Uplift. The position of these basin deeps, the deepest on the Colorado Plateau, indicates a strong overall northward tilt of the plateau block toward the trend of the Mendocino zone. The deep of the Denver Basin is located where the 40th parallel intersects the east side of the Southern Rockies. The deepest part of the Salina Basin is near the 40th parallel but is centrally located between the gentle Cambridge Arch on the west and gentle west side of the Nemaha Uplift on the east. The deep of the Forest City Basin is at the intersection of the east-west zone and the faulted, steep, east side of the north-south trending Nemaha Uplift. The deep of the present Appalachian Basin is also at 40°N, where that parallel crosses the west front of the Valley and Ridge Province of the Appalachians. The Illinois Basin, the only exception, is some miles to the south of the 40th parallel. The deep of this basin may, however, prove the rule, for it is near the juncture of a possible